A Miniature Biosensor Based On Guided Wave Technology And An Acousto Optic Tunable Filter

Phase II Final Report

Contract # DAAM01-95-C-0025

Issued by

United States Army CBDCOM

Technical Monitor:	Mr. Roy Thompson Edgewood Research Development & Engineering Center Aberdeen Proving Ground
Contractor:	Brimrose Corporation of America 5020 Campbell Blvd., Suite E Baltimore, MD 21236
Investigators:	Dr. Sean X. Wang Dr. Vladimir Pelekhaty Dr. Jolanta I. Rosemeier Mr. Keith Li Mr. Robert Scheerer
Effective Date of Contract:	March 13, 1995
Contract Expiration Date:	March 13, 1997 (Extension to June 27, 1997)
Reporting Period:	March 13, 1995 - June 25, 1997

brimrose
5020 campbell blvd., baltimore, maryland 21236 usa
tel: 410/931-7200 ● fax: 410/931-7206 ● e-mail: office@brimrose.com

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (*Leave Blank*)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	970730	(Final) 0950313-970730

4. TITLE AND SUBTITLE
A Miniature Biosensor Based On Guided Wave Technology and Acousto-optic Tunable Filter

5. FUNDING NUMBERS
DAAM01-95-C-0025
Req. # CRDECO-1995-0196

6. AUTHOR(S) Sean X. Wang, P.I.
Vladimir Pelekhaty, Jolanta I. Rosemeier,
Keith Li, Robert Scheerer

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Brimrose Corporation of America
5020 Campbell Blvd., Suite E
Baltimore, MD 21236

8. PERFORMING ORGANIZATION REPORT NUMBER
Final

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army CBDCOM
Bldg. E4455
Aberdeen Proving Ground, MD 21010-5423

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Distribution unlimited; available for public release

12b. DISTRIBUTION CODE

13. ABSTRACT (*Maximum 200 words*)

In Phase II of this SBIR program Brimrose has researched, designed, and built a miniature solid-state biosensor module based on an acousto-optic tunable filter (AOTF) and embedded into an optical immunodetection system. It offers the combined advantages of compact size, lightweight. low operating power. long life time, ruggedness without moving parts, high speed with random wavelength access capability and zero tuning hysteresis. In addition, Brimrose has conducted in depth investigations, both theoretical and experimental, of Integrated-Optic AOTF (IOAOTF) structures for biosensor applications. The final comparative analysis of performance characteristics of both integrated-optic and bulk AOTFs had revealed significant advantages of the bulk one both in throughput and spectral tuning range. This resulted in the selection of the bulk type AOTF as the spectral selective element of the optical biosensor module embedded into the immunosensor system. Applications for this product include: general fluorescence spectroscopy; biochemical engineering and biotechnology; remote sensing; industry process and quality control; monitoring of analyte concentrations in experimental animals and in cell cultures; and water monitoring of environmental pollutants and contaminants.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Acousto-optic tunable filter, Miniature solid-state biosensor module, Integrated-optic AOTF (IOAOTF), Fluorescence Spectroscopy	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Same as Report

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

REF D

A Miniature Biosensor Based On Guided Wave Technology And An Acousto Optic Tunable Filter

PHASE II CONTRACT

FINAL REPORT - TABLE OF CONTENTS

A Miniature Biosensor Based On Guided Wave Technology And An Acousto Optic Tunable Filter

PHASE II CONTRACT

TABLE OF CONTENTS - PAGE 2

A Miniature Biosensor Based On Guided Wave Technology And An Acousto Optic Tunable Filter

PROJECT SUMMARY

Brimrose Corporation of America
5020 Campbell Blvd.
Baltimore, MD 21236

The US Government is currently concerned about the increased use of biological weapons, particularly by Third World countries. It is therefore necessary to strengthen the US Army's biological defense capability by developing advanced technology for reconnaissance, detection, and identification (RDI). Unfortunately, there is no adequate biosensor readily available for hand held battle field applications. The technical objective of this SBIR program is therefore to develop a miniature optical immunosensor for biodetection.

In Phase II of this SBIR program Brimrose has researched, designed, and built a miniature solid-state biosensor module based on an acousto-optic tunable filter (AOTF) and embedded into an optical immunodetection system. It offers the combined advantages of compact size, lightweight. low operating power. long life time, ruggedness without moving parts, high speed with random wavelength access capability and zero tuning hysteresis. In addition, Brimrose has conducted in depth investigations, both theoretical and experimental, of Integrated-Optic AOTF (IOAOTF) structures for biosensor applications. The final comparative analysis of performance characteristics of both integrated-optic and bulk AOTFs had revealed significant advantages of the bulk one both in throughput and spectral tuning range. This resulted in the selection of the bulk type AOTF as the spectral selective element of the optical biosensor module embedded into the immunosensor system.

Applications for this product include: general fluorescence spectroscopy; biochemical engineering and biotechnology; remote sensing; industry process and quality control; monitoring of analyte concentrations in experimental animals and in cell cultures; and water monitoring of environmental pollutants and contaminants.

The technical objective of this Phase II effort was to develop a miniature optical immunosensor for biodetection. This goal was pursued in two parallel ways. In one way research and investigation of suitable structures for an integrated-optic AOTF biosensor were performed. An integrated-optic AOTF has been fabricated and its performance characteristics were tested and analyzed. In another way, a bulk optic AOTF was further improved and optimized as a potential spectral selective element of miniature biosensor module. The comparative analysis of throughput and spectral tuning range of both integrated-optic and bulk optic AOTFs has revealed significant advantages of the bulk optic AOTF. Hence the later one was selected as a spectral selective element of the optic biosensor module. Besides of AOTF it incorporates a green diode-pumped solid-state Nd-doped laser with intracavity second harmonic generation for the excitation of the fluorescence, Si PIN photodiode with high gain and low noise preamplifier for fluorescence detection, collimating and focusing optics, and newly designed miniature fiber optic probe, which allows to test extremely small volumes of liquid sample material -- down to one cubic millimeter. The biosensor control system consists of an embedded 386 computer with a solid state disk and communication ports. A host PC computer (such as laptop) may network with the biosensor system for data transmission, program loading and display. The performance specifications for biosensor module are summarized in the following table:

Brimrose/CBDA Biosensor Module

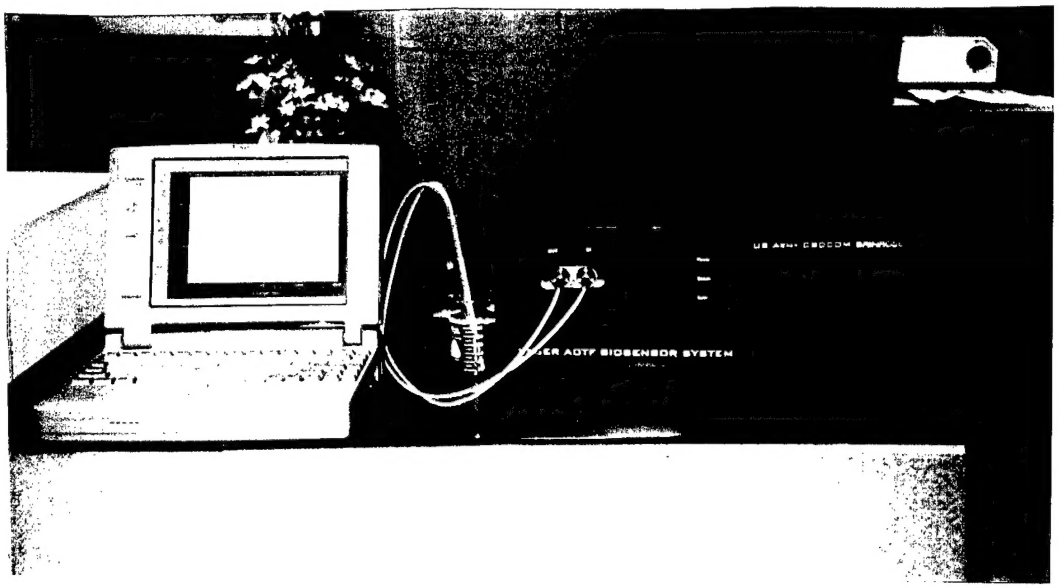
Physical Dimensions	(12.7x5.6x2.4 cm)
Weight	250 gm
Power Requirements	1 Watt
Power Supply	battery or AC powered
Spectral Resolution	1.7 nm @ 543 nm, 2.7 nm @ 633 nm
Wavelength Range	450-700 nm
Sensitivity	-90 dbm
Scan Speed	4,000 wavelengths/second
Scan Increment	0.1-10 nm
Wavelength Accuracy	±0.25 nm
Noise Rejection	>1000:1
Software	Luminar Windows software package to acquire, process, and view spectral data

A Miniature Biosensor Based On Guided Wave Technology And An Acousto Optic Tunable Filter

EXECUTIVE SUMMARY

Brimrose Corporation of America
5020 Campbell Blvd.
Baltimore, MD 21236

In this Phase II SBIR program, Brimrose has developed and built an optical immunosensor system for biodetection. This system contains an embedded biosensor module, which employs an acousto-optic tunable filter (AOTF) to perform fluorescence spectral analysis and, to the best of our knowledge, is **the world's smallest biosensor** of its kind. The sensor is compact, lightweight, rugged with **no** moving parts, and the system displays rapid spectral analysis with random wavelength access capability.



Photograph of the optical immunosensor system with the embedded miniature biosensor module.

1.0 IDENTIFICATION OF THE SIGNIFICANCE OF OPPORTUNITY

1.1 Introduction

The increasing use of biological weapons among Third World countries has imposed a potential threat to US Army Forces. It is necessary to strengthen the US Army's biological defense capability by developing advanced technologies for reconnaissance, detection and identification (RDI). Unfortunately, there is currently no adequate biosensor readily available for battle field applications. Innovative methods and sensor designs are therefore required.

In this PhaseII SBIR program, Brimrose proposed to research, build and develop a novel miniature fluorescence spectrometer for biodetection. The biosensor is based on the utilization of miniature acousto-optic tunable filters (AOTFs). The optical spectral analysis is accomplished via anisotropic acousto-optic interaction. The fast wavelength tuning over a wide bandwidth is made possible by an acoustic dynamic grating controlled by simply changing the driven RF frequency.

This solid-state fluorescence spectrometer module offers the advantages of light weight (< 200 gm), small size, high sensitivity, rapid spectral analysis (4000 wavelengths per second) and reliability and ruggedness with no moving parts.

1.2 State of Art Limitations

In order to analyze the detected fluorescence spectrum, some kind of monochromator or wavelength filtering device has to be employed. It is desirable for this device to be compact, light weight and programmable with fast and accurate random access. For military applications the device must also be rugged without the need for frequent maintenance or recalibration. Several different technologies may be used, but each exhibit some serious limitations.

The scanning diffraction grating spectrometer is a well proven tool in many types of spectroscopy. However, it needs to be frequently recalibrated and mechanically adjusted, is susceptible to mishandling and vibration, and takes a finite time to scan between wavelengths.

The newer grating spectrometers using a fixed grating and detector array are much faster and better suited to industrial environments, requiring less maintenance and/or recalibration. However, the selected wavelength is still a function of a precise geometrical arrangement between the grating and detector. Vibration or mishandling can thus cause "blurring" of the image on the array, which translates into reduced performance.

Bandpass Filters simply do not offer the flexibility for most real world applications. A separate filter is needed for each wavelength data point.

Electro-optic filters exhibit the same filter response profile as AOTFs but achieve wavelength tuning by electro-optically changing the material birefringence. Since material birefringence is only weakly tunable this results in a restricted tuning range (several nm).

Fabry - Perot interferometer filters such as air gap and liquid filled devices feature narrow passband widths but they also exhibit multiple transmission lines throughout the optical spectrum, which inhibits their use in spectroscopy applications. Additionally, their mechanical wavelength tuning incur prolonged measurement times in comparison to AOTFs, which severely restricts the efficiency of data collection.

Fourier Transform (FT) Spectrometers are capable of excellent resolution and sensitivity. Unfortunately, since their operation involves precision translation of mirrors, their performance is also very sensitive to environmental factors - such as vibrations and shock. In addition, they are relatively slow since they can only collect and compute spectral data over the entire combined Bandpass of the instrument.

Clearly, none of these technologies offer the required combination of speed, ruggedness, miniaturization, flexibility, reliability and hands-off operation required in battle field applications. Hence, the interest is in alternative technologies such as the AOTF.

1.3 Acousto-Optic Tunable Filters (AOTFs)

The AOTF is based on the acoustic diffraction of light in an anisotropic medium [1-4]. It consists of a piezoelectric transducer bonded to a birefringent crystal. When the transducer is excited by an applied RF signal, acoustic waves are generated in the medium. The propagating acoustic wave produces a periodic modulation of the index of refraction. This provides a moving phase grating which, under proper conditions, will diffract portions of an incident light beam. For a fixed acoustic frequency, only a limited band of optical frequencies can satisfy the phase-matching condition and be cumulatively diffracted. As the RF frequency is changed, the center wavelength of the optical passband is changed accordingly so that the phase matching condition is maintained.

Anisotropic acousto-optic diffraction involves a 90 degree rotation of the polarization plane of the diffracted wave. If an AOTF is sandwiched between crossed polarizers, only those optical components within the AOTF passband will be polarization converted. Wavelength components outside of the passband are either blocked or spatially separated. This is illustrated in figure 1.

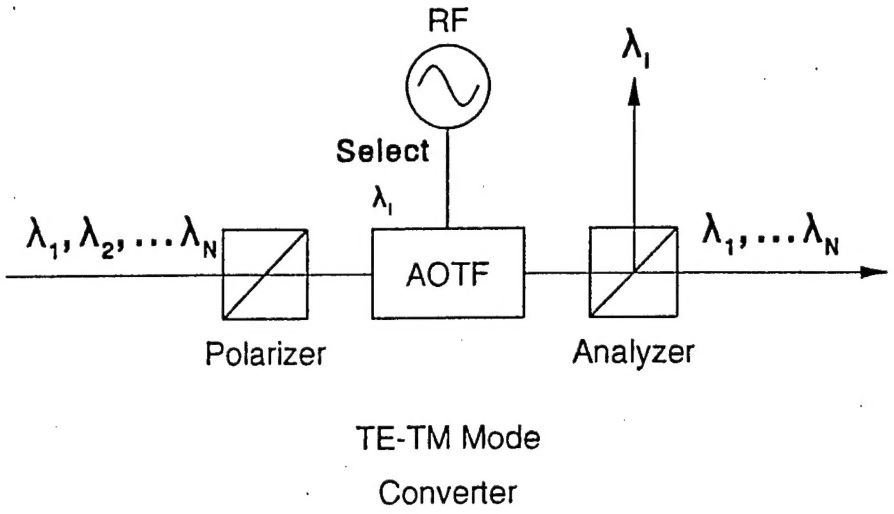


Figure 1. Schematic drawing of an AOTF. It acts like a TE-TM mode converter sandwiched between a pair of crossed polarizers. Wavelength tuning can be accomplished by changing the RF frequency.

1.4 Special Features of AOTFs

Since the AOTF is a rapidly tunable filter, it is ideal for use as the monochromator at the heart of a fluorescence spectrometer. In the following, we shall discuss how the various features of an AOTF benefit fluorescent spectrometer applications. These benefits make the AOTF more than just another technology for consideration, but the ideal tool for fluorescence spectroscopy.

Rugged and Compact Device/No Moving Parts AOTFs are solid state devices and are both compact and rugged. Clearly a device with no moving parts is much more insensitive to damage in military and industrial applications. Just as important, short of actually breaking the device, vibrations and shocks will not affect the wavelength calibration.

High Efficiency With Low Power Consumption: AOTFs are highly efficient devices with transmission at the selected wavelength as high as **98%**. Unlike a "classical" monochromator in which the entrance/exit slits define the spectral resolution and limit the overall optical throughput, the spectral resolution of an AOTF is independent of the optical aperture and optical throughput can be high. High efficiency translates directly into lower operating power, higher sensitivity and therefore faster data acquisition. In addition, the acoustic drive power requirements of an AOTF are typically from several hundreds of

milliwatts to **less than 100 mW** in some specially designed devices.

Broad Tuning Range/High Resolution: High resolution **(~1nm)** can be achieved with a broad tuning range **(350-900nm)**. In fluorescent spectroscopic measurements, out of band transmission should be kept to a minimum, preferably zero. The AOTF excels in this area, with out of band transmissions as low as 10^{-5} for some devices.

Fast Speed/Random Access When the RF frequency is changed, the limiting factor in changing the wavelength is the time it takes for the acoustic wave to fill the optical aperture - typically from **several µs to tens of µs**. This means that entire spectra can be scanned at very high speed, or discrete wavelengths may be accessed at rates of **tens to hundreds of KHz**, even when separated by hundreds of nanometers.

Repeatability/Calibration: The AOTF is an all solid state device with no moving parts. For a given device geometry, the transmitted wavelength is determined only by the frequency of the applied RF, which can be generated with digital precision. This means that an AOTF based spectrometer can be easily self calibrated by merely changing the RF frequency. Since fluorescent spectroscopy applications usually require measurements at multiple wavelengths, short and long term wavelength repeatability are highly advantageous. As an example, a typical Brimrose bulk TeO_2 AOTF has a wavelength repeatability error of less than ± **0.05 nm**.

Multiple Simultaneous Measurements: The AOTF can be programmed to simultaneously pass multiple wavelengths because the grating is formed by an acoustic wave rather than by fabrication. By driving an AOTF at multiple acoustic frequencies, multiple wavelengths can be measured simultaneously. This property can be exploited to measure multiagents and implement powerful matched filtering algorithms which can greatly increase the sensitivity to important chemical spectra whilst decreasing the sensitivity to background noise.

Built-in Solid State Chopper For Lock-In Amplification: The intensity of the selected light is controlled electronically and can be rapidly modulated. This makes the AOTF ideal for use with a lock-in (phase and frequency sensitive) amplifier for low level light detection under strong ambient light. This scheme is extremely beneficial in a open space sampling system.

Computer Control/Integration One of the most useful features of the AOTF is its high degree of controllability or programmability. In AOTFs, the RF synthesizer is interfaced directly to a microprocessor or computer. This enables an AOTF based spectrometer to be programmed to scan or access different wavelengths very rapidly, and even to change the output intensity at those wavelengths. In use, therefore, it is easily integrated into almost any computer controlled measurement system.

2.0 TECHNICAL APPROACHES

AOTFs can be categorized into two main kinds, bulk crystal devices and integrated optical waveguide structures.

2.1 Non-collinear TeO_2 AOTFs

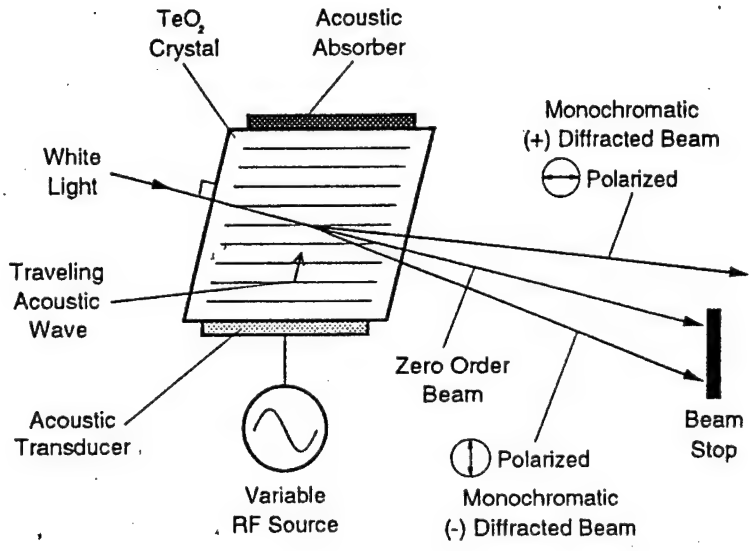


Figure 2. Schematic representation of a TeO_2 non-collinear AOTF.

In the visible wavelength region, the most common choice of material for the AOTF is TeO_2, which is very efficient with a relatively large acousto-optic figure of merit and is transparent up to 5.5 μm. TeO_2 based AOTFs are usually designed in a so called non-collinear configuration - the acoustic and optical waves propagate at quite different angles through the crystal. Figure 2 is a schematic representation of a non-collinear TeO_2 AOTF. The approximate **tuning relation** between the center frequency of the acoustic wave and the optical passband center wavelength is given by

$$f = \frac{v_a \Delta n}{\lambda} \sqrt{\sin^4 \theta_i + \sin^2 2\theta_i} \qquad (1)$$

where θ_i is defined as the angle between the optical axis and the incident light. The **peak transmission** T_o is given by the ratio of diffracted light to that of incident light.

$$T_0 = \sin^2\left(\frac{\pi^2 M_2}{2\lambda_0^2} P_d L^2\right)^{1/2} \quad (2)$$

where M_2 is a normalized acousto-optic figure of merit, L is the interaction length and P_d is the acoustic power density;

$$P_d = \frac{P(acoustic\ power)}{H(width) \times L(length)} \quad (3)$$

Also, like a Bragg cell, the tunable filter is a linear device. Therefore multiple passbands can be generated simultaneously by using more than one excitation frequency. The **spectral resolution** of the tunable filter is given by;

$$\Delta\lambda = \frac{1.8\pi\lambda_0^2}{bL\sin^2\theta_i} \quad (4)$$

where b is the dispersion constant given by

$$b = 2\pi\left(\Delta n - \lambda_0 \frac{\partial \Delta n}{\partial \lambda_0}\right) \approx 2\pi\Delta n \quad (5)$$

An additional consideration is the FOV of the tunable filter. This parameter is proportional to the total amount of light which may be collected through the tunable filter using an AOTF. Any scene can be decomposed into an angular spectrum of plane waves. For a given wavelength of operation, the resolution of the imaging system is ultimately determined by the largest angle plane wave which the optical system can accommodate. To first order, the **angular FOV** is given approximately by;

$$\Delta\theta_i \approx n\sqrt{\frac{\pi\lambda_0}{\Delta n L |F_\theta|}} \quad (6)$$

where

$$F_\theta = 2\cos^2\theta_i - \sin^2\theta_i \quad (7)$$

The FOV is typical in the order of a few degrees. The angular separation between the zeroth order undiffracted beam and either of the diffracted beams is given by

$$\Delta\theta = \Delta n \sin 2\theta_i \quad (8)$$

As shown in eqn. 1, the wavelength of light that is selected by this diffraction can therefore be varied simply by changing the applied RF frequency. As indicated in figure 2, the diffracted light intensity is directed into two physically separated first order beams, termed the (+) and (-) beams. These beams are orthogonally polarized, which is utilized

in certain applications. Also, the two orthogonally polarized beams separate when they exit from the crystal and the angle of the deflected beam does not vary appreciably with changes in the optical wavelength. This implies that only a single fixed detector is necessary during a spectral scan.

To use the AOTF as a tunable filter in the conventional way, a beam stop is used to block the undiffracted broadband light and the (+) and/or (-) monochromatic light is directed to the experiment. The angle between the beams is a function of device design but is typically a few degrees (see eqn. 8). The bandwidth of the selected light depends on the device design and the wavelength of operation, and is typically several nanometers in the VIS/NIR region. Transmission efficiencies are high (up to 98%), with the intensity divided between the (+) and (-) beams. Another useful and unique feature of the AOTF, as shown in eqn. 2 is its ability to precisely and rapidly adjust the intensity of the diffracted (filtered) light by varying the RF power (P_d).

2.2 Integrated-Optic Acousto-Optic Tunable Filters (IOAOTFs)

The same kind of opto-acoustic interaction also exists in thin film waveguide in which both optical and acoustic field are confined within a very thin (several micrometers) surface guiding layer [6-8].

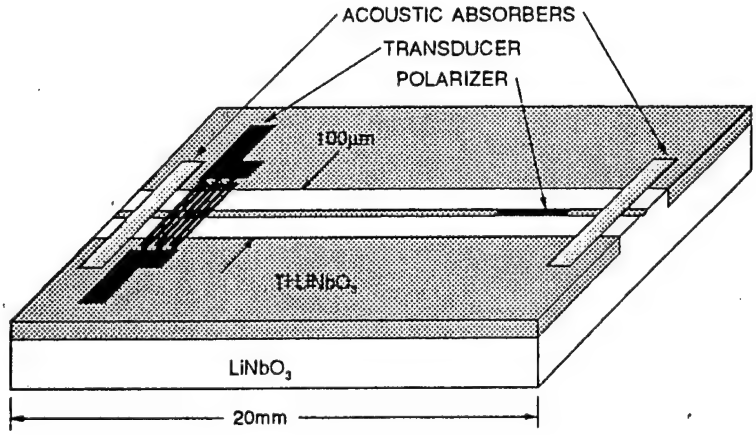


Figure 3. Schematic of a simple integrated-optic AOTF.

A simple IOAOTF design is shown in figure 3. The optical beam, confined to propagate in a titanium diffused optical waveguide, interacts with an acoustic beam, launched by an interdigital transducer (IDT). Surface acoustic waves (SAW) generated by the IDT cause a periodic modulation of the $LiNbO_3$ substrate's index of refraction. This acts as a diffraction grating which mode converts a narrow band of optical frequency components, determined by the acoustic frequency, into the orthogonal polarization state. Thus, by preparing the input beam into one of the substrate modes (polarization states) then

filtering can be achieved at a wavelength determined by the acoustic frequency. Acoustic absorbers are employed to prevent reflections from the end facets, which can interfere with the device filtering, and acoustic waveguides are diffused on to the surface of the substrate to confine the acoustic beam, and thereby reduce the RF drive power requirements. The optical polarizer serves to reject the unfiltered wavelength components.

The phase-matching condition for the collinear acousto-optic interaction is given as follows:

$$|k_{TM}| - |k_{TE}| = |K| \tag{9}$$

Where k_{TM} and k_{TE} are the wave vectors of the TE and TM optical modes, respectively, and K is the wave vector of the SAW. The corresponding acoustic frequency f_a is simply given by:

$$f_a = \frac{V_a}{\lambda_o}|n_{TE} - n_{TM}| \tag{10}$$

Here v_a is the SAW velocity, λ_o is the vacuum optical wavelength and n_{TM} and n_{TE} are the effective mode indices of the TM and TE modes respectively. It is evident that the acoustic frequency is dependent upon the birefringence of the optical waveguide. For example, this leads to a SAW frequency of approximately 500 MHz for λ_o=0.63 μm in a Ti:$LiNbO_3$ waveguide. However, by an additional proton exchange the birefringence can be modified yielding a SAW frequency between 1-300 MHz.

The peak transmission at phase matching can be presented as follows:

$$T_o = \sin^2[\eta_o(\frac{L^2}{W})P_a]^{1/2} \tag{11}$$

Here P_a is the total acoustic power, W and L are the width and length of the interaction region and η_o is a mode conversion factor which includes the photoelastic coupling coefficients and the overlap integral of optical and acoustic field. In comparison to the bulk AOTF, the IOAOTF can achieve high efficiency with lower power. This is due to the fact that the diffraction limitations of interaction length (L) can be eliminated by a channel waveguide for both guided optical waves and the SAW. Hence, the IOAOTF can achieve the same level of efficiency with much less RF power.

In this integrated optoelectronics approach, components including laser, photo-detector, acoustic transducer and micro-processor may be integrated in a single substrate. The device can be very compact, light weight (in the order of **several grams**), and low in power consumption (**as low as several mW**). The device is potentially low cost and optoelectronic integration will allow for easy mass production similar to electronic integrated circuits.

3.0 DEVICE DESIGN, FABRICATION, AND TESTING

3.1 The Non-collinear TeO_2 AOTF

The miniature TeO_2 non-collinear AOTF has been designed with optimized aperture and transmission characteristics. The specifications for this device is summarized below:

Selected Specifications of Brimrose AOTF.

Material	TeO_2
Spectral Range	450-700 nm
Optical Aperture	7x7 mm
Acceptance Angle	3 deg.
Access Time	20 μsec
Tunability	0.1 nm
Repeatability	0.05 nm
Spectral Resolution	<1.5 nm @ 500 nm
Diffraction Efficiency	90%@633 nm

The AOTF of this specifications was designed, fabricated, and tested. This AOTF was found to exhibit the optimum performance for biosensor module application due to its high optical throughput. Figure 4 shows the orientation and physical dimensions of this AOTF. Figure 5 plots the AOTF's optical resolution vs. optical wavelength. As it already was shown in eq. 4, the resolution is proportional to the square of the optical wavelength. Figure 6 is the tuning curve (RF frequency vs. the optical wavelength). Figure 7 plots the relation of acceptance angle vs. wavelength. The manufacturing flow chart for the AOTF is illustrated in Figure 8.

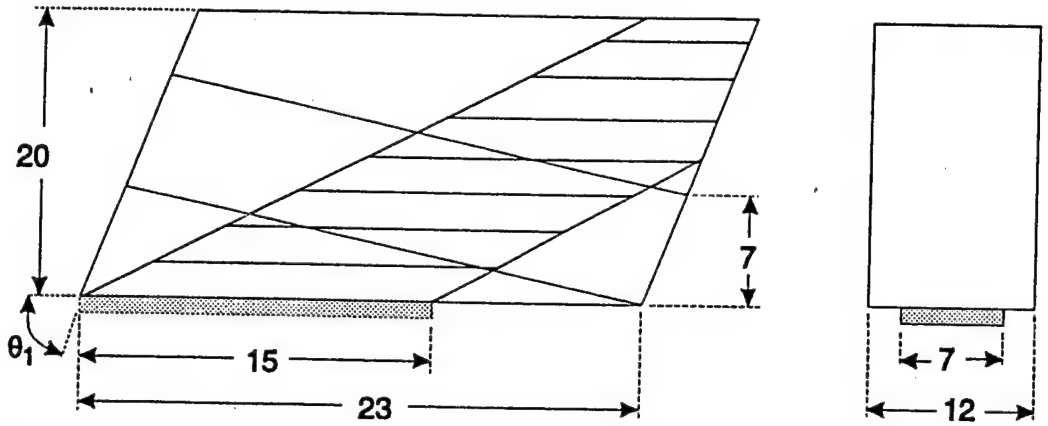


Figure 4. Shapes and physical dimensions of the designed AOTF. Dimensions are in millimeters

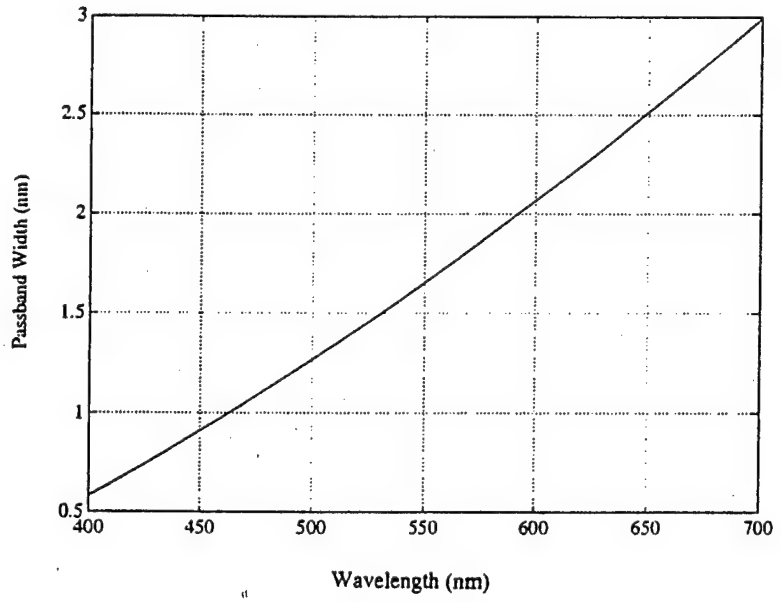


Figure 5. The optical transmission band resolution vs. optical wavelength.

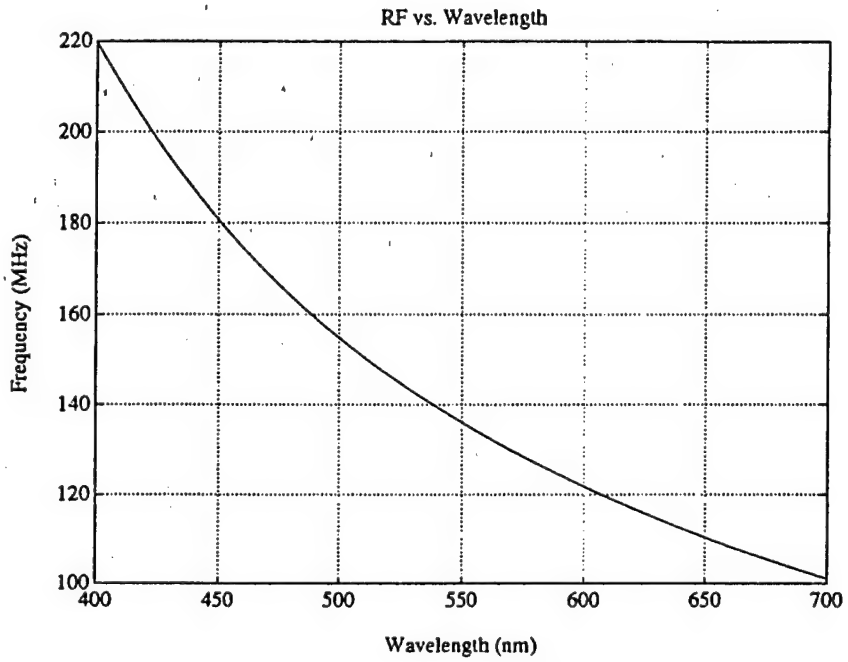


Figure 6. The AOTF tuning curve (RF frequency vs. transmitted center optical wavelength).

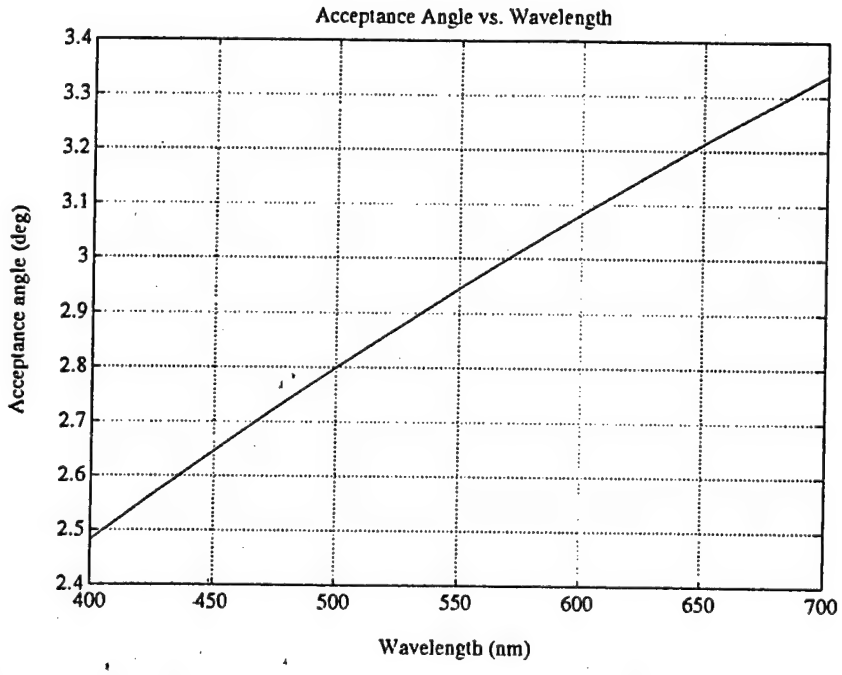


Figure 7. The acceptance angle of the AOTF as a function of optical wavelength.

AOTF Manufacturing Process

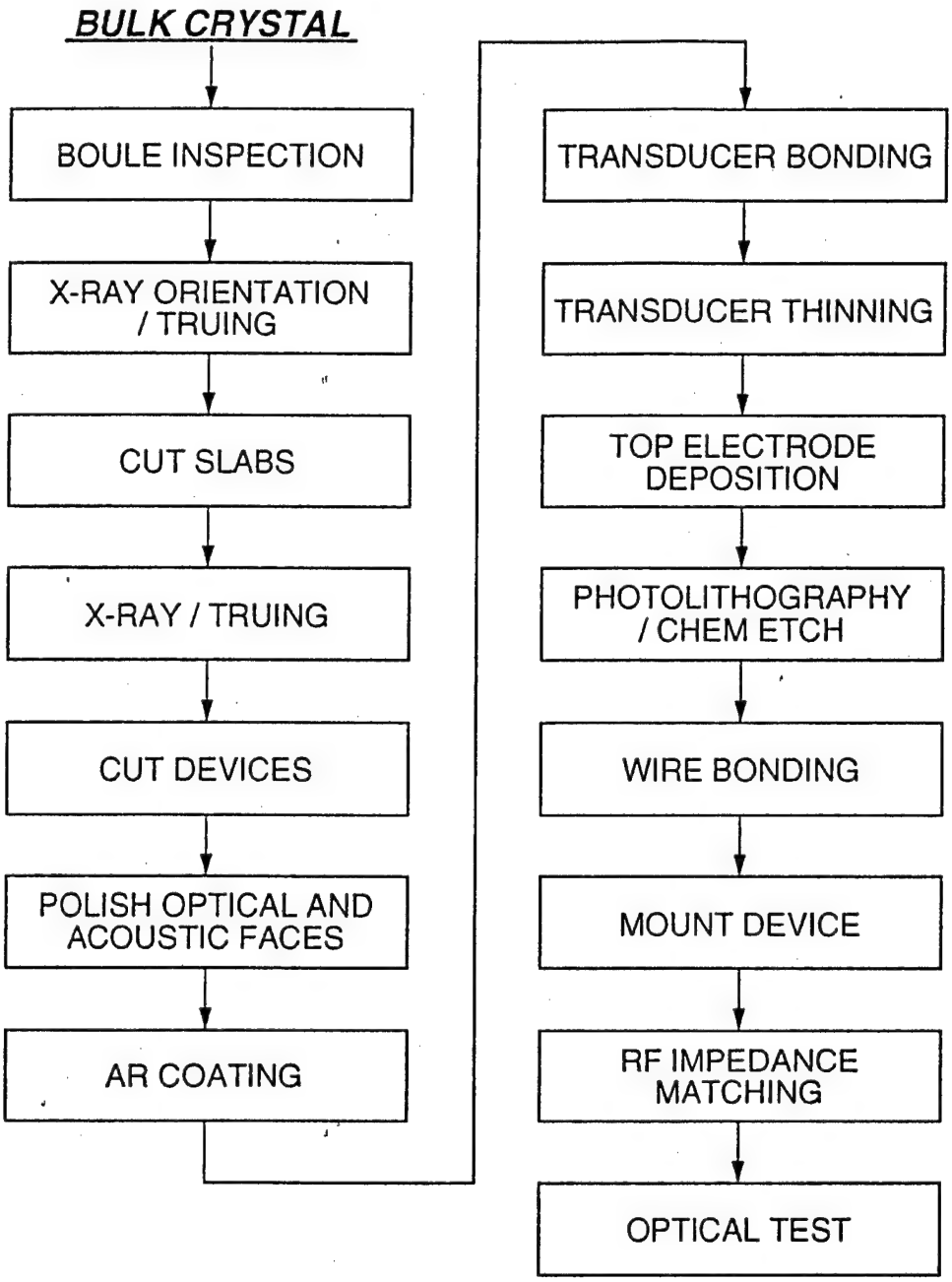


Figure 8. AOTF fabrication flow chart.

3.1.1 Optical Substrate Preparation

The bulk TeO_2 crystal was x-ray oriented using a Laue back reflection technique. The crystal boule was cut with the proper orientation for the AOTF. The orientation included repeated Laue, grinding, blocking, and milling of the TeO_2 crystal to achieve acceptable angular alignment of the crystal axes. This detailed fabrication procedure, along with the final Laue exposures, was recorded for subsequent quality control monitoring. After orientation, the crystal was blocked to enable accurate sawing along the desired axis. Using this technique, a slab was produced which was suitable for further fabrication. In the next step of the fabrication process the bonding and optical surfaces of the cut sample were prepared. Figure 9 is the Laue pattern of the [110] surface of the 3x3mm aperture device.

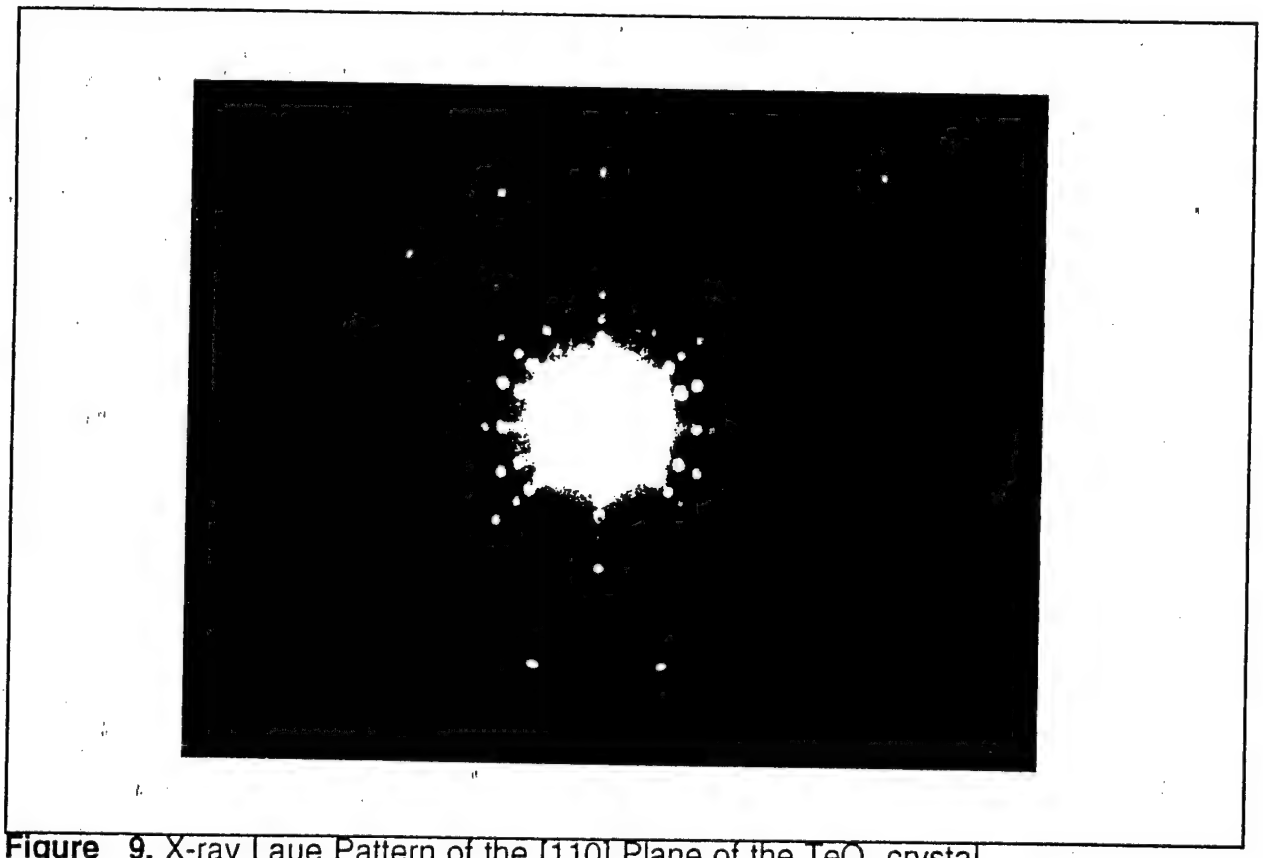


Figure 9. X-ray Laue Pattern of the [110] Plane of the TeO_2 crystal.

3.1.2 Shear Mode Transducer

The shear mode $LiNbO_3$ transducer was concurrently produced for bonding to the AOTF optical substrate. This component was prepared in slab form. A slab 1/4"x2" was cut from an existing oriented boule and the bonding faces were polished as one unit. The particle motion was aligned appropriately in the proper direction.

3.1.3 Bonding Process

After deblocking, the substrate strips were bonded with a shear mode transducer. Thinning of the shear mode devices ended with the frequency of 100-220 MHz as the desired acousto-optic objective. The final thickness of the transducer was calculated based upon the following formula:

$$d = \frac{V_a}{2 f_a} \tag{18}$$

where d, V_a and f_a are the thickness, acoustic velocity and desired frequency. Since the acoustic frequency in the shear mode $LiNbO_3$ is 4800m/s, the required transducer thickness for 135 MHz operations is 17.8 μm. During the thinning process, the transducers were closely monitored with a Taylor-Hobson Tally Surf. Resolution of the instrument is 400 Angstroms. After the optical substrate strip was lapped down to a frequency of 85-170 MHz, the strip was sawed into devices to expose the optical faces. The optical faces were ground and polished.

3.1.4 Electrical Impedance Matching/Optical Testing

At this stage, the device is mounted to a mechanical supporting base and the top electrode is wire bonded to a microwave strip line. The impedance matching is performed by using an on-line microwave network analyzer, which displays the Smith Chart as well as the standing wave ratio of the device under test over the whole frequency range of interest. State of the art impedance matching techniques are employed to match extremely low impedances (> 0.5 ohm) of big area (1-2 cm^2) transducers to the standard 50 ohm generator throughout more than octave of the frequency range. Among them are both common LC ladder networks and more advanced quarter-wave and transmission line transformers. Figure 10 represents the Smith Chart of the AOTF matched to the 50 ohm generator. The quality of the impedance matching is so that less than 10% of RF power is reflected back from the AOTF to the generator, as the plot for reflection coefficient shows in Figure 11.

Finally, the optical parameters are tested. The efficiency and resolution are measured by using lasers of different wavelength as a light source. By sweeping the frequency of an RF source around a specific the central frequency, corresponding to the specific laser wavelength (with infinitesimal linewidth in comparison to the filter response of an AOTF), the peak optical transmissions as well as the bandpass can be measured. The examples of such test data are shown in Figures 12 and 13 for green (543 nm) and red (633 nm) He-Ne lasers. The transmission sidelobes are well suppressed at around -10 dB below the main transmission peak and the acousto-optic conversion efficiency is approximately 90%. The FWHM passband width is 0.35 MHz at 543 nm and 0.4 MHz at 633 nm, which corresponds to optical resolutions of 1.7 nm and 2.7 nm respectively. This agrees well with the theoretical prediction of 1.6 nm at 543 nm and 2.35 nm at 633 nm, as shown in Figure 5.

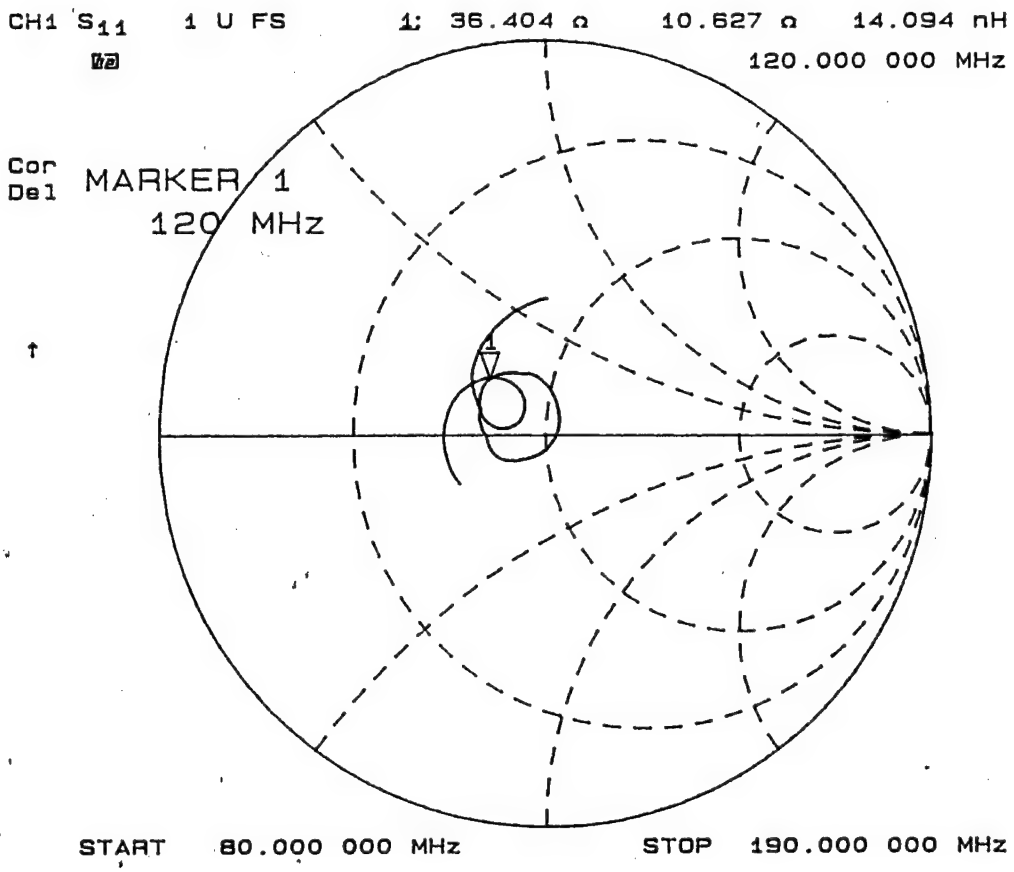


Figure 10. Impedance Smith Chart for the bulk-optic TeO_2 AOTF.

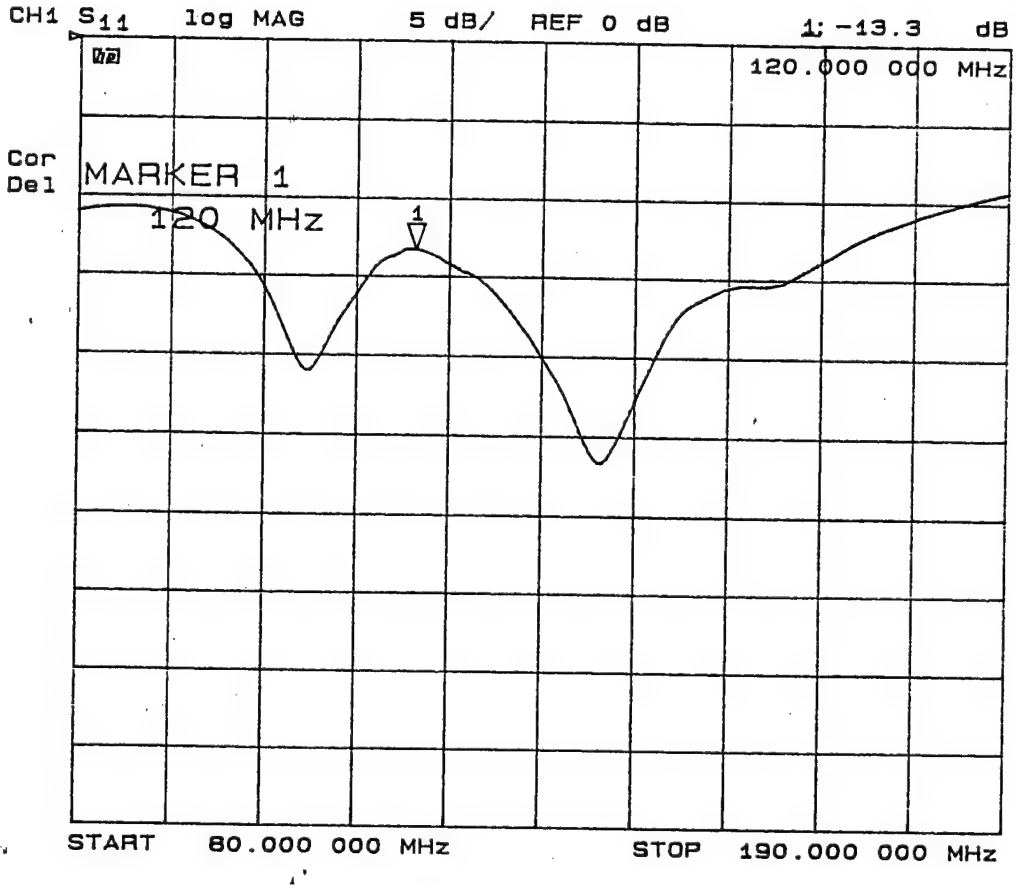


Figure 11. RF power reflection coefficient for the bulk-optic TeO_2 AOTF.

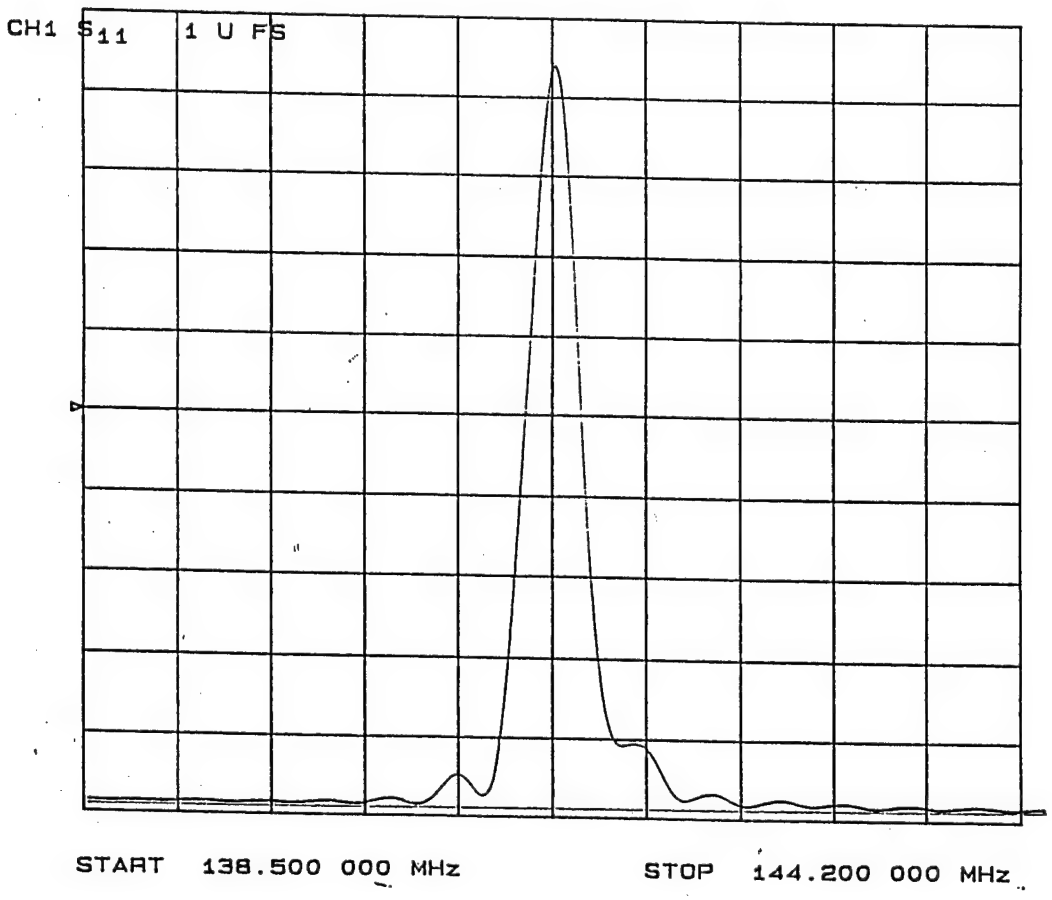


Figure 12. Response profile of the bulk-optic TeO_2 AOTF at 543 nm.

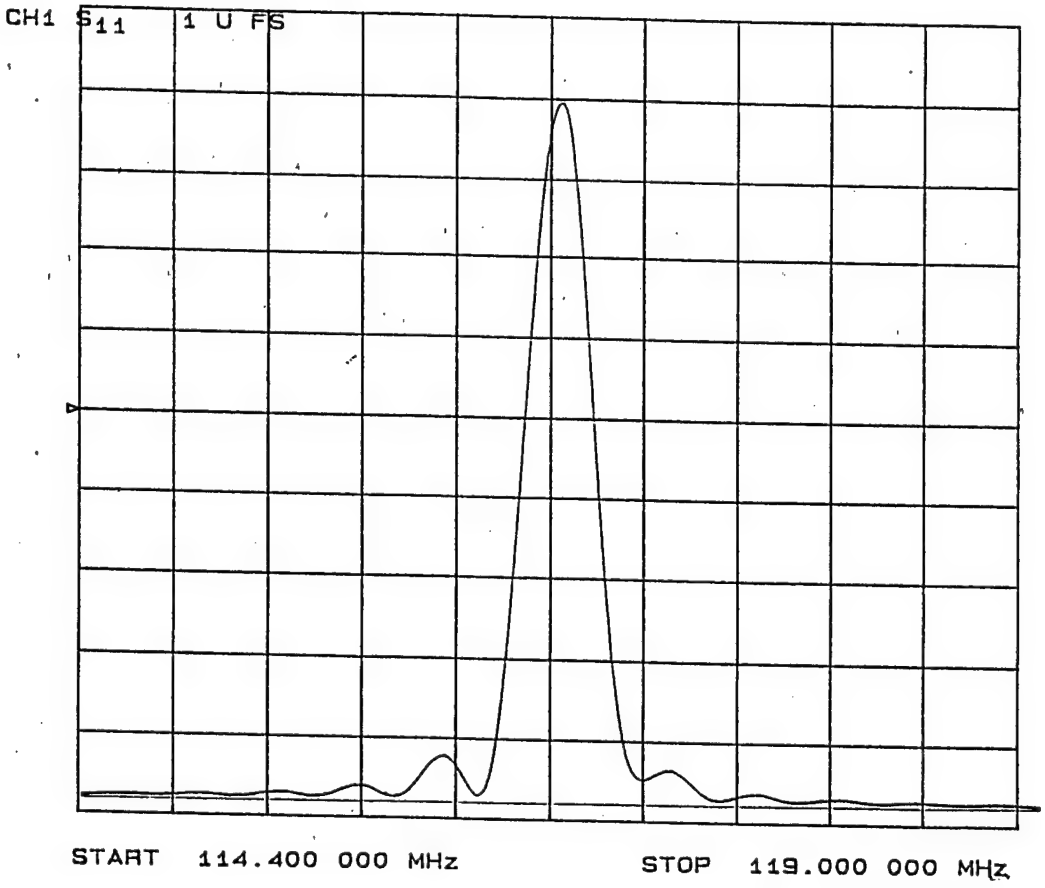


Figure 13. Response profile of the bulk-optic TeO_2 AOTF at 633 nm.

3.2 The $LiNbO_3$ Integrated-Optic AOTF

The integrated-optic AOTF, designed during this Phase II program, was fabricated and tested. The .device consists of 25x3x1 mm $LiNbO_3$ substrate on which titanium optical and acoustic waveguides are thermally indiffused. The optical waveguide was designed to be 8 μm wide to facilitate efficient optical fiber to waveguide coupling, whilst the acoustic waveguides channel was chosen to be 100 μm wide to provide a high degree of acoustic confinement. An aluminum interdigital transducer (IDT), of aperture equal to the width of the acoustic waveguide, is photolithographically deposited on the crystal surface to generate surface acoustic waves required for acousto-optic diffraction.

3.2.1 Generation of Surface Acoustic Waves

Maximum electromechanical conversion efficiency occurs when the IDT is driven at a frequency f_a which corresponds to the acoustic velocity V_a divided by the period of the IDT Λ:

$$f_a = V_a / \Lambda_a ,$$

because at this frequency the period of the generated acoustic wave is equal to the period of IDT and it becomes resonant at this frequency. The spacings between the fingers of the IDT are chosen to satisfy the periodicity criterion for phase matching of the mean wavelength to be filtered. Conventional IOAOTF is designed to filter out the infrared radiation around 1.53 μm, which results in the central frequency equal to about 178 MHz and IDT period being equal to about 20 μm , so IDT is having 5 μm fingers and 5 μm spaces between them. Impedance matching of the interdigital transducer is performed in a similar manner to the bulk-optic AOTF devices, and its Smith Chart is shown in Figure 14.

3.2.2 Optical Testing

The efficiency and resolution of the integrated-optic AOTF was measured in similar manner to the bulk-optic AOTF, by sweeping the frequency of an RF source around a specific laser wavelength. The device does not contain integrated polarizers, therefore external polarizers were required to separate the filtered wavelength components from the undiffracted light. The measured response profile for 1523 nm He-Ne laser is represented in Figure 15, showing 2 nm resolution and almost 100% diffraction efficiency at about 100 mW of RF drive power.

The next Figure 16 illustrates the tuning range of the integrated-optic AOTF. It shows the part of the spectrum of the broad band surface emitting LED with the center wavelength around 1.5 μm. The tuning range of the IOAOTF is about 80 nm FWHM, which is determined by the strongly resonant multifinger electrode structure of the IDT. Such a narrow tuning range (just only 5% of the central wavelength) poses as great

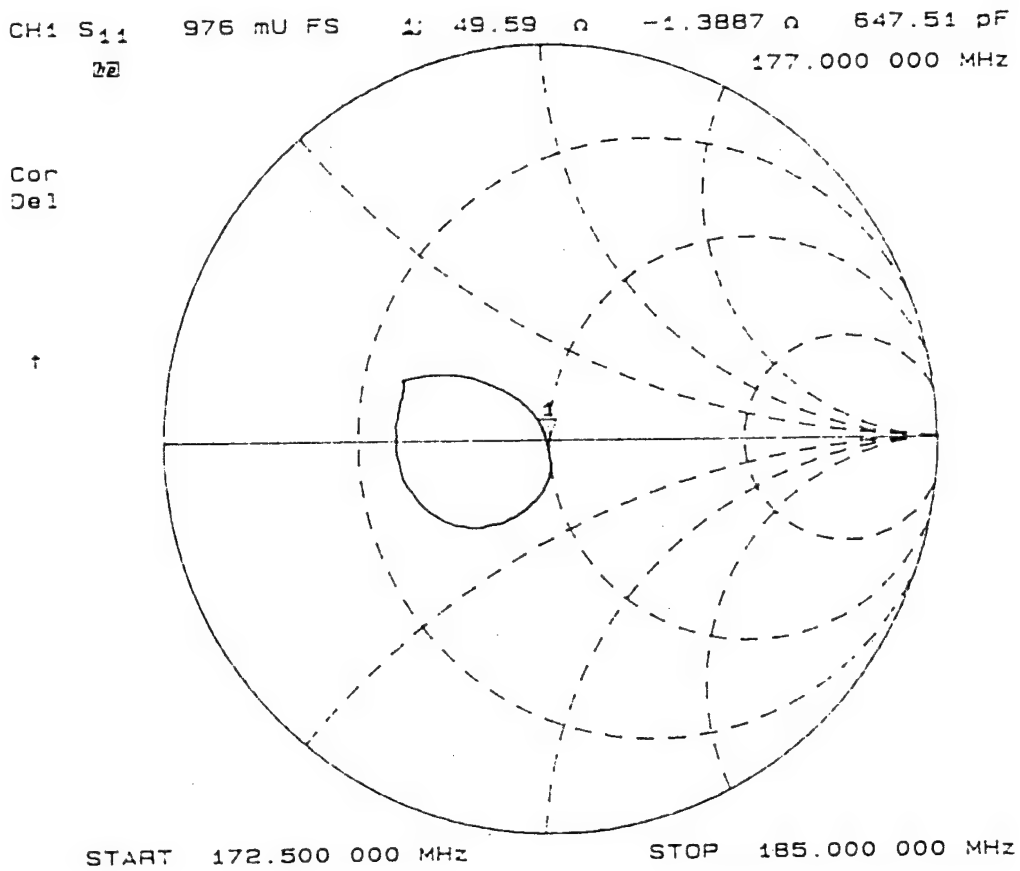


Figure 14. Impedance Smith Chart for the Integrated-Optic AOTF

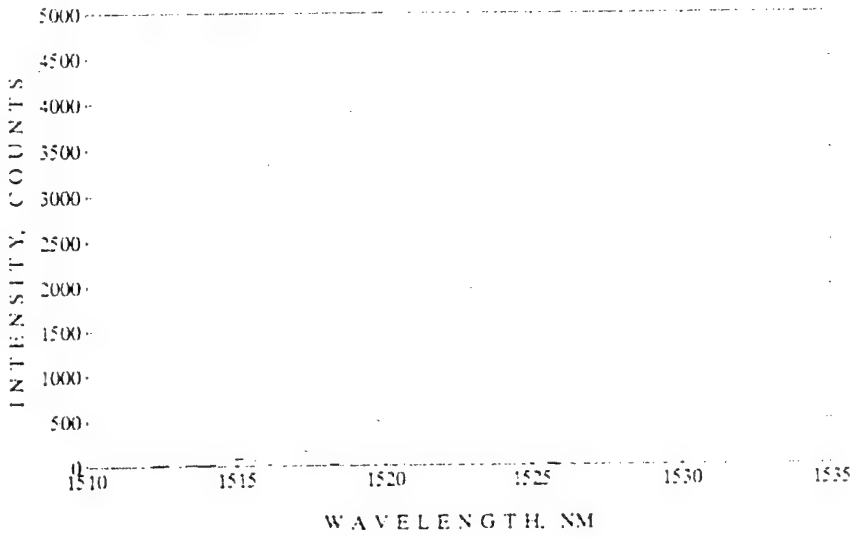


Figure 15. Response profile of the Integrated-Optic AOTF for 1523 nm He-Ne laser.

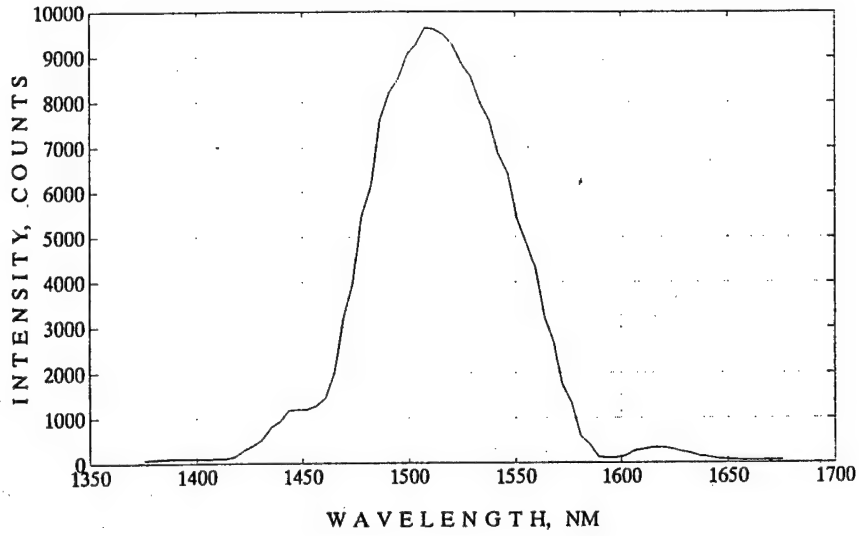


Figure 16. Tuning curve of the Integrated-Optic AOTF.

drawback in fluorescence applications, where much broader spectral tuning ranges are required.

The device under test was operating at central wavelength about 1.5 μm, but fluorescence spectra of the biological samples are located mostly in the visible part of the optical spectrum. To design an IOAOTF for visible rang it would take a waveguide only 3 μm in diameter and RF frequency should be swept from 400 MHz to 1000 MHz, which alone would require the submicrometer photolithography to deposit the proper IDT. Optical crossection of the IOAOTF of the order of 10 μm^2 compared to the 0.5 cm^2 of the bulk-optic AOTF makes the throughput of the IOAOTF negligibly small compared to that of the bulk-optic one.

All the above mentioned drawbacks of the IOAOTF prevented us from it's implementation as spectral selective element of the fluorescence optical biosensor module. Hence, the bulk-optic AOTF was chosen as spectral selective element of the fluorescence optical biosensor module.

4.0 THE MINIATURE AOTF BIOSENSOR MODULE

The miniature biosensor module, based on a non-collinear TeO_2 AOTF, has been designed, fabricated, and tested. The biosensor module employs optical fibers to direct excitation laser light to, and to collect fluorescent light from, the sample material. An electronic control subsystem, which includes digital and analog boards, computer hardware and software package, has been modified from a Brimrose Luminar 2000 NIR spectrometer and provides control, data acquisition and data processing to the biosensor module.

4.1 System Layout

The system approach adopted for implementing the biosensor is illustrated in Figure 17. It consists of two separate modules, biosensor module and electronic control module. The biosensor module has been miniaturized into a small package of 12.7x5.6.x2.4 cm and weighs 250 gm.

In the module, the green light from the Nd-doped diode pumped solid-state laser with intracavity second harmonic generation is coupled into a multimode optical fiber and delivered to the sample probe. This green laser light excites the fluorescence of the sample material. The fluorescence light is collected and coupled into another multimode fiber and delivered via it to the AOTF for spectral filtering and to Si PIN photodiode connected to the high gain and low noise preamplifier for fluorescence detection. The detected signal is relayed to the electronic control subsystem for lock-in amplification, digitization, and further processing.

4.2 Miniature Probe

During the feasibility experiment performed in Phase I standard industrial optical fluorescence probe was used with the optical biosensor module. It employs two multimode fiber optic collimator to direct the excitation light to the sample and to collect the fluorescence light. Those two collimators have 5-6 mm diameters and are oriented at 90° to each other to minimize the feedback of light from the excitation source. This standard probe was not optimized for the optical fluorescence measurements of biological sample material. First, it's 5-6 mm beam diameter has all the excitation laser power spread throughout it cross-section, resulting in low power density. Another drawback is that it is bulky and cumbersome, having 1" diameter and being 7" long, it requires at least 100 milliliters of liquid sample material for the test.

To eliminate these drawbacks, the new miniature fire optic probe was designed and fabricated. It is sketched in Figure 18. It has to multimode optical fibers 0.6 mm in diameter, one for excitation and another for collection of the fluorescence, oriented at

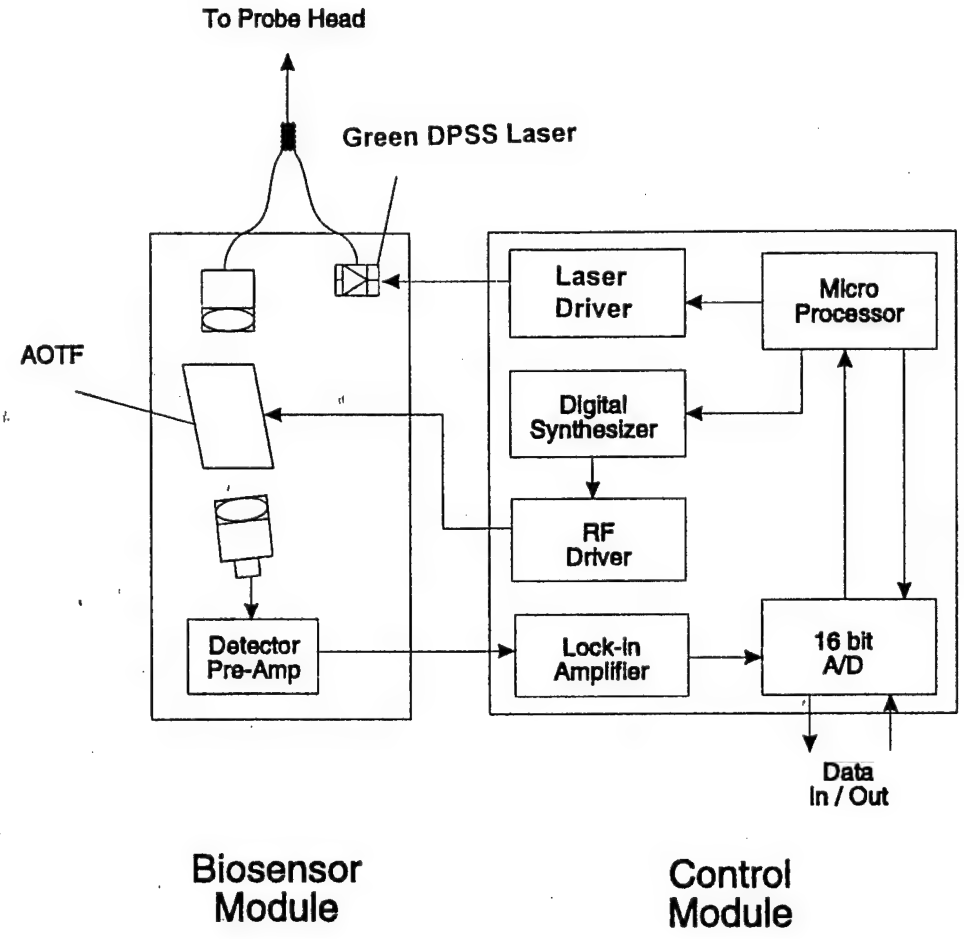


Figure 17. System illustration of the miniature biosensior.

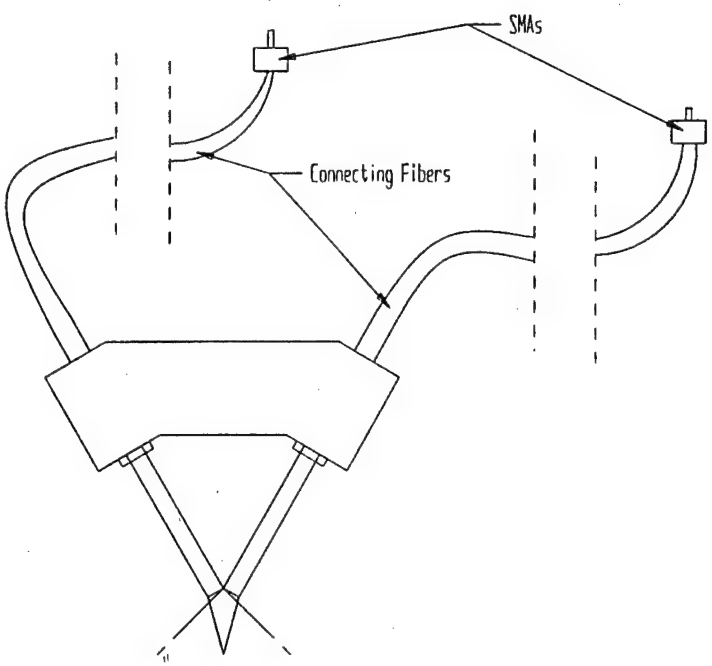


Figure 18. Sketch of the newly designed miniature fiber optic probe for the test of small volumes of liquid biological sample material.

an angle of 60^{o} to each other. Approximately 10 times smaller beam diameter results in 100 times higher power density and, consequently, higher excitation efficiency. Another advantage of it is that it allows to test a single droplet of the liquid sample material, about 1 mm^3, which makes it very suitable for the intended optical fluorescence measurements of the biological substances.

In conclusion, Figure 19 represents the experimental fluorescence spectrum of Rhodamine laser dye dissolved in water with the concentration of several parts per billion. Its shows excellent performance and sensitivity of the optical fluorescence sensor system.

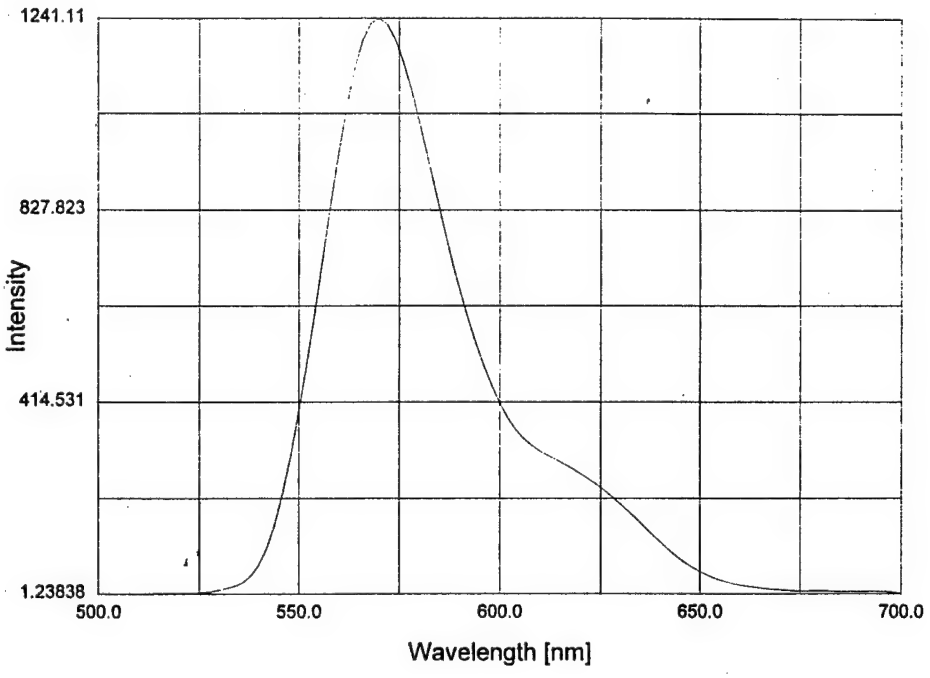
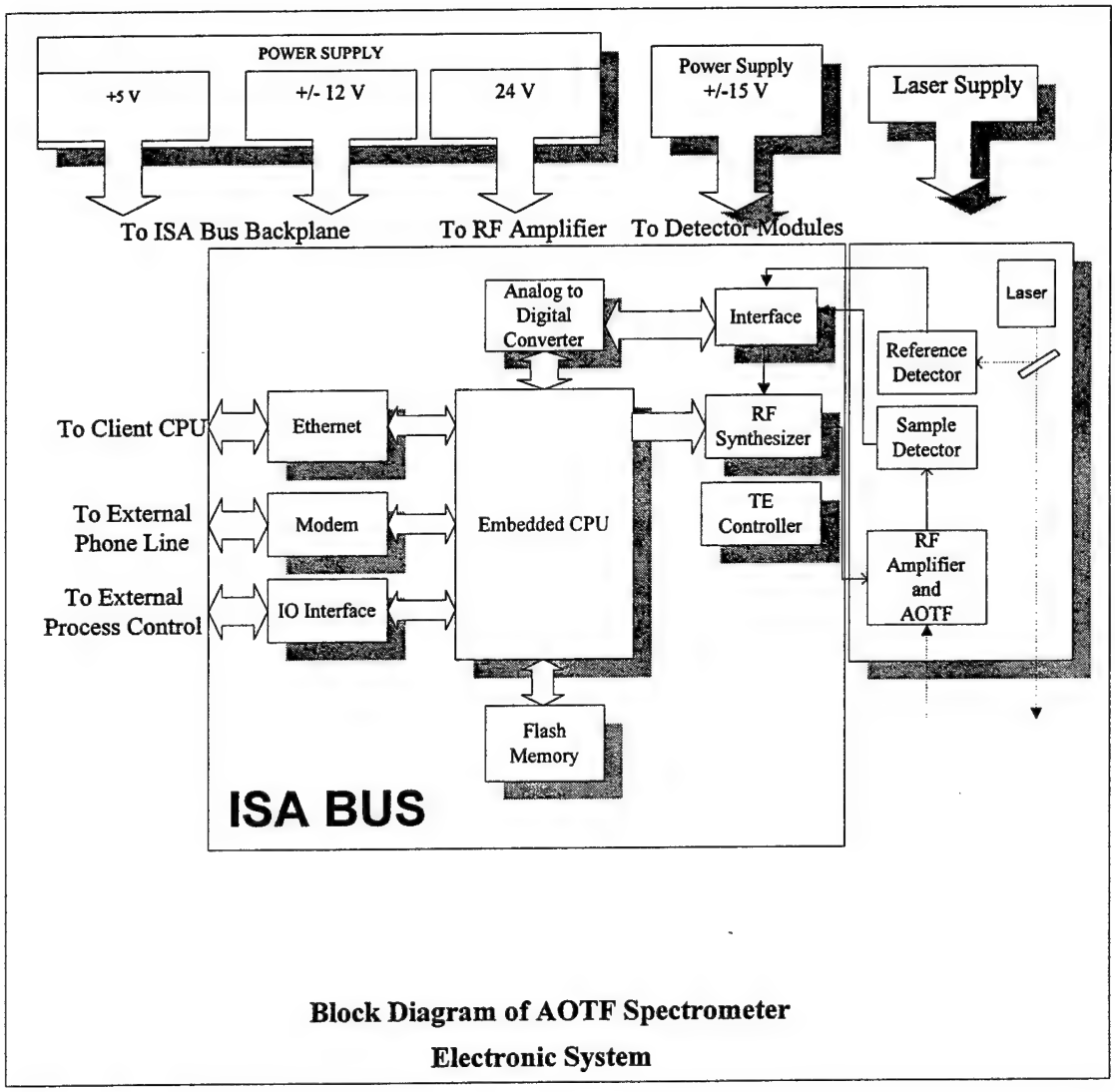


Figure 19. Fluorescence spectrum of Rhodamine laser dye dissolved in water with the concentration of several parts per billion.

5.0 ELECTRONIC SYSTEM

Introduction

The Electronic system for the spectrometer is based on the existing commercial NIR spectrometer produced by Brimrose with modifications made to the optical assembly for accommodating a laser as the sample illuminating source and the method by which the reflected wavelengths are collected.



Block Diagram of AOTF Spectrometer

Electronic System

Fig. 20

Operation

As shown in Fig. 20 the system is made up of several components each dependent on the other for total operation of the system. Data entered into the software package located on the "client:" PC is sent via an ethernet connection to the "host" PC located within the spectrometer. This data includes wavelength data, gain control, etc. The host

PC using embedded application software translates the wavelength data to the equivalent RF needed by the AOTF to select the wavelength. The RF card is then programmed for each frequency within the wavelength range in frequency increments determined by the wavelength step size.

Depending on the RF signal applied the AOTF allows only the corresponding wavelength within the spectral range to pass through to the visible detector. The visible detector converts the detected wavelength to an electrical equivalent of its reflected intensity. A sweep across the spectral range thus provides an equivalent electrical image of the reflected intensities of all wavelengths across the entire range that can be electronically processed.

The function of each component and its part in the operation of the unit is outlined, beginning with commercial components and followed by components designed and manufactured by Brimrose Corporation.

Commercial Components

CPU with Solid Disk Drive - The spectrometer is driven by a 386 or 486SX processor (with co-processor option) responsible for all facets of data collection and diagnostic processes of the entire instrument. The CPU dictates which RF frequency relates to a desired wavelength, sets the RF card for the desired frequency, processes reflectance, transmittance and diagnostic data obtained from the analog-to-digital card and provides for remote access through an ethernet or modem connection. The motherboard contains serial, parallel, keyboard, floppy, and fixed disk interfaces allowing the unit the capability of being able to be programmed and operated independently of a "client" PC.

ROM/RAM Disk Drive - The ROM/RAM Disk Drive serves as the boot drive (A:\) for the CPU and contains DOS version 5.0, and the application and communications software necessary to run the spectrometer. Total RAM disk space can be from 360Kbytes to 6Mbytes.

16-Bit Ethernet Card - The ethernet card allows for communication between the spectrometer ("host" computer) and a remote PC ("client") using a standard TCP/IP network. A user of the system is thus capable of remotely monitoring all data processed by the spectrometer as well as remotely performing system diagnostics in order to verify the performance of the unit. The 16-bit ethernet card is fully compatible with Novell's NE2000 driver, transfers data at a rate of 10Mbits/sec and can support either 10BaseT (Twisted pair) and 10Base2 (Thin-BNC) cabling.

Display Card - A VGA display card is available for when the spectrometer is used in stand alone operation. This allows for the connection of an external monitor directly to the spectrometer when needed.

Fax/Modem Card - A 14400/28800 bps fax/modem card allows for communication between the spectrometer and a remote PC across a telephone line. Like the ethernet card it provides the capability to remotely monitor data processed by the spectrometer and to run remote diagnostics.

Data Acquisition Card - The function of the data acquisition card is to convert all analog signals from the analog interface card to their digital equivalent so that they can be processed by the CPU. The card is preset to accept sixteen single ended analog inputs and to provide eight digital outputs. The digital outputs are used to provide gain settings on the analog interface card.

Brimrose Components

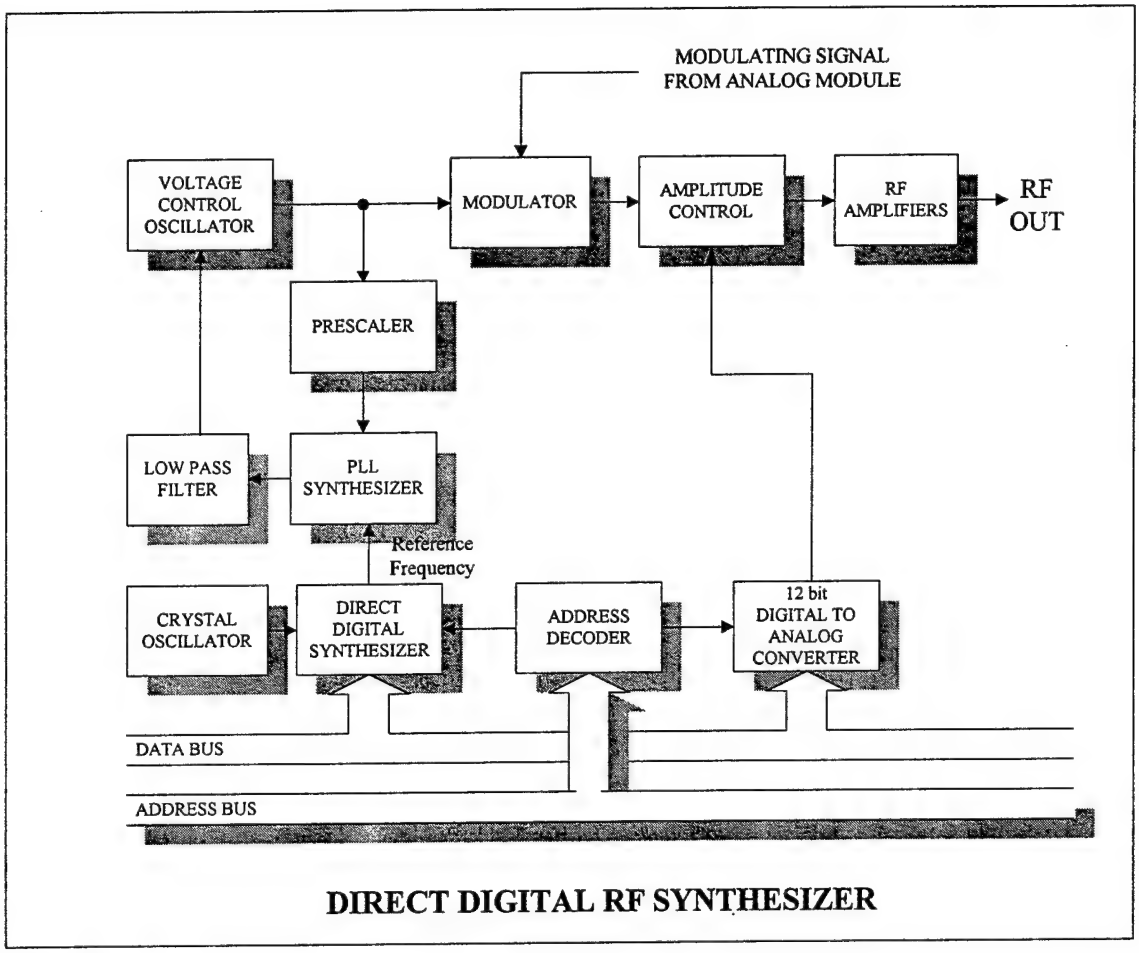


Fig. 21

RF Synthesizer (Fig. 21) - The RF synthesizer generates an RF signal in the range of 35 MHz to 80 MHz with 30 mHz resolution and an output power of 40 mW. The card consist of three parts a Direct Digital Synthesizer (DDS) for providing a reference frequency range from which the output RF is derived, a Phase Locked Loop (PLL), and a 12 bit D/A converter for amplitude control of the of RF frequency. The CPU supplies the synthesizer with binary information representing the reference frequency and amplitude of the output RF signal.

Sample Detector Module (Fig. 22) - Bio-Chemical substances demonstrate different flourescence properties within the visible spectral region. The sample detector module contains a visible detector which detects these flourescence properties as variations in the light energy across the spectral region as light is emitted by a bio-chemical substance. The output of the visible detector provides a linearly related electrical image corresponding to the variations in light energy detected.

Reference Detector Module (Fig. 22) - Variations in the laser light source intensity tend to decrease the signal-to-noise ratio of the spectrometer. The reference detector module samples a part of the laser beam and ratios it against the sampled spectrum for reduced noise levels.

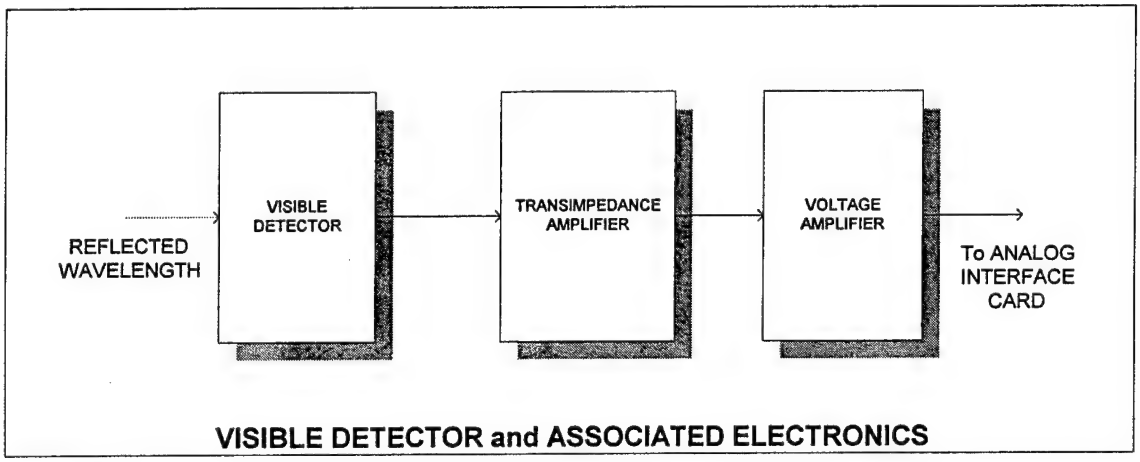


Figure 22

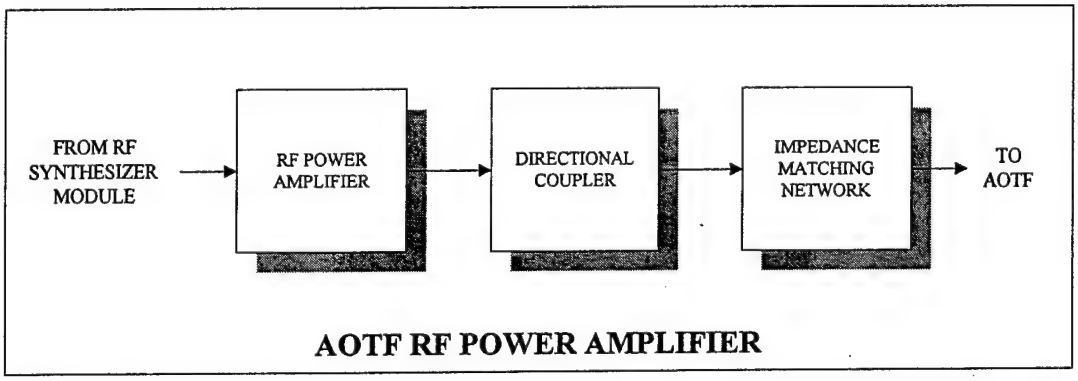


Figure 23

RF Power Amplifier (Fig. 23) - The power amplifier provides the AOTF device with the RF power need for its correct operation. The amplifier amplifies the 40 mW RF signal from the RF synthesizer to 2 W. Impedance matching circuitry between the power amplifier and the AOTF device ensures maximum RF power transfer to the AOTF.

Analog Interface Card (Fig. 24) - The Analog Interface card provides analog signal processing of the signals received from the visible detectors. This includes filtering of the signals to remove unwanted frequency components, digital gain and lock-in amplification for removal of noise introduced by components in preceding stages. The card also contains electronic modules for diagnostic sensors used within the unit. This allows the performance of the unit to be checked and compared with its normal operating specifications.

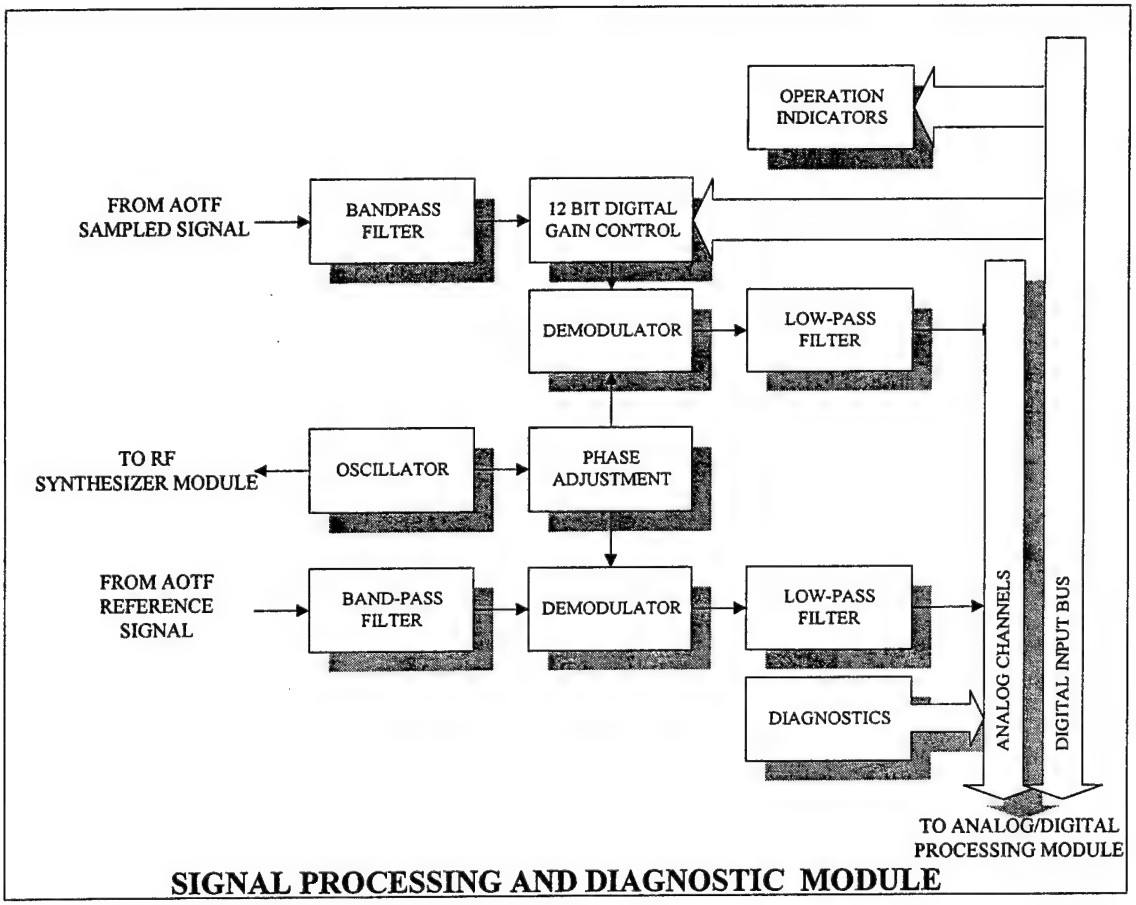


Fig. 24

Component Specifications:

CPU Card

- **CPU:** 486DX4-100
- **Bus Interface:** ISA bus
- **Data Bus & Processing Ability:** 32-bit
- **DRAM memory:**
  1MB to 32 MB, (4MB installed in system).
- **IDE hard disk drive interface:**
  Supports up to two IDE hard disk drives.
- **Floppy disk drive interface:**
  Supports up to two floppy disk drives, 5¼" (360 KB and 1.2 MB) and/or 3½" (720 KB and 1.44 MB).
- **Bi-directional parallel port:**
  Configurable to LPT1, LPT2, LPT3 or disabled.
- **Serial ports:**
  One-serial RS-232 port, one serial RS232/422/485 port. Ports can be individually configured as COM1, COM2 or disabled.
- **Max power required:** +5 V @ 2 A
- **Operating temperature:** 32 to 140° F (0 to 60°C)
- **Size:** 7.3"(L) x 4.8" (W)

ROM/RAM Disk Drive

- **Disk sizes:** 360 KB to 6MB (1.5MB used in system)
- **Size:** 7.3" (L) x 3.9" (W)
- **Max power required:** +5 V @ 1 A
- **Operating temperature:** 32 to 140° F (0 to 60°C)

Ethernet Card

- **Data rate:** 10 Mbits/sec
- **Cable:**
  Thin-cable RG-58 AU coaxial cable or UTP cable. (UTP cable used in system)
- **Compatibility:** Compatible with Novell NE2000
- **Distance:**
  For Thin-cable network: 925 meters (3,035 feet) with repeaters and 185 meters (607 feet) without repeater.
  For UTP cable network: 100 meters for link segments and 500 meters for multiple hubs.
- **Nodes per segment:** 30 (Thin Network)
- **Max. power required:** +5 V at 1 A.
- **Size:** 159mm (L) x 70mm (W)
- **Operating temperature:** 0 to 45°C

Display Card

- **Resolution:** Up to 1280 x 1024
- **Memory:** Up to 1 Mbyte of display memory.
- **Bus Interface:** 16 bit video bus interface

Fax/Modem Card

- **Data speeds:** 28,800 14,400

Data Acquisition Card

- **Bus interface:** ISA Bus
- **Number of analog inputs:**
  8 differential, 16 single ended, 16 psuedo-differential. (16 single ended channels used in unit)
- **Resolution:** 16 Bits
- **Analog full scale range:**
  +/- 10V, +/- 5V, +/- 2.5V and 0 to 10V. (Unit set for 0 to 10V)
- **Number of digital inputs/outputs:** 8 bits
- **Digital input level:** TTL
- **Size:** 3.9" x 6.0"
- **Operating Temperature:** 0 to 55°C
- **Max power required:** +5 V @ 1.4 A

RF Synthesizer

- **Frequency range:**
  40 to 80 Mhz / 60 to 120 Mhz with < 1 dB attenuation
- Frequency Resolution: 30 mHz
- **Frequency accuracy and stability:** Accurate to 0.01 %
- **Output Impedance:** 50 ohms
- **RF power output:** +16 dBm (40mW)
- **RF power output resolution:** 12 bits
- **Max. power required:** +5 V, +12 V
- **Size:** 7.25" x 4.55"

RF Power Amplifier

- **Frequency range:** 40 to 550 Mhz
- **RF output power:** +33 dBm (2 Watts) min @ 1 dB compression
- **Power gain:** 17.7 dB
- **Max. power required:** 24V@ 500 mA

Analog Interface Card

- **Digital gain:** Digitally controlled gain of 1, 2, 4 and 8
- **Max. power required:** +5 V, +/-12 V
- **Size:** 7.5" x 4.55"

Detector Module

Spectral response: 400 nm to 700 nm
Max. power required: +/-15 V

6.0 SOFTWARE DESCRIPTION

Overview

The Luminar software package for Windows lets you acquire, process, and view spectral data sets collected from the Luminar 2000 spectrometer. The package consists of several application programs that use standard Windows graphical user interface (GUI) to simplify data acquisition and processing routines.

The functions covered by the package include:

- data acquisition and monitoring,
- viewing, editing and post-processing data,
- file manipulation (translation to other formats, merging/splitting, truncating),
- data set graphing.

This release of the manual covers DDEMENU.EXE version 1.66 and LUMSERV.EXE version 1.62. The details of the programs will be described below.

System requirements

The following hardware and software are needed to run the Luminar Windows software:

- a 386-based (or better) PC-compatible personal computer equipped with at least 8MB RAM, a VGA display adapter, an Ethernet network adapter and at least 12MB free hard disk space,
- Windows 3.1/3.11 or Windows 95 installed and running.
- For Windows NT, 32 MB is strongly recommended for optimal performance

To achieve best results, we recommend to run the software on a Pentium PC equipped with a fast hard drive, 16MB minimum of RAM and an accelerated display adapter.

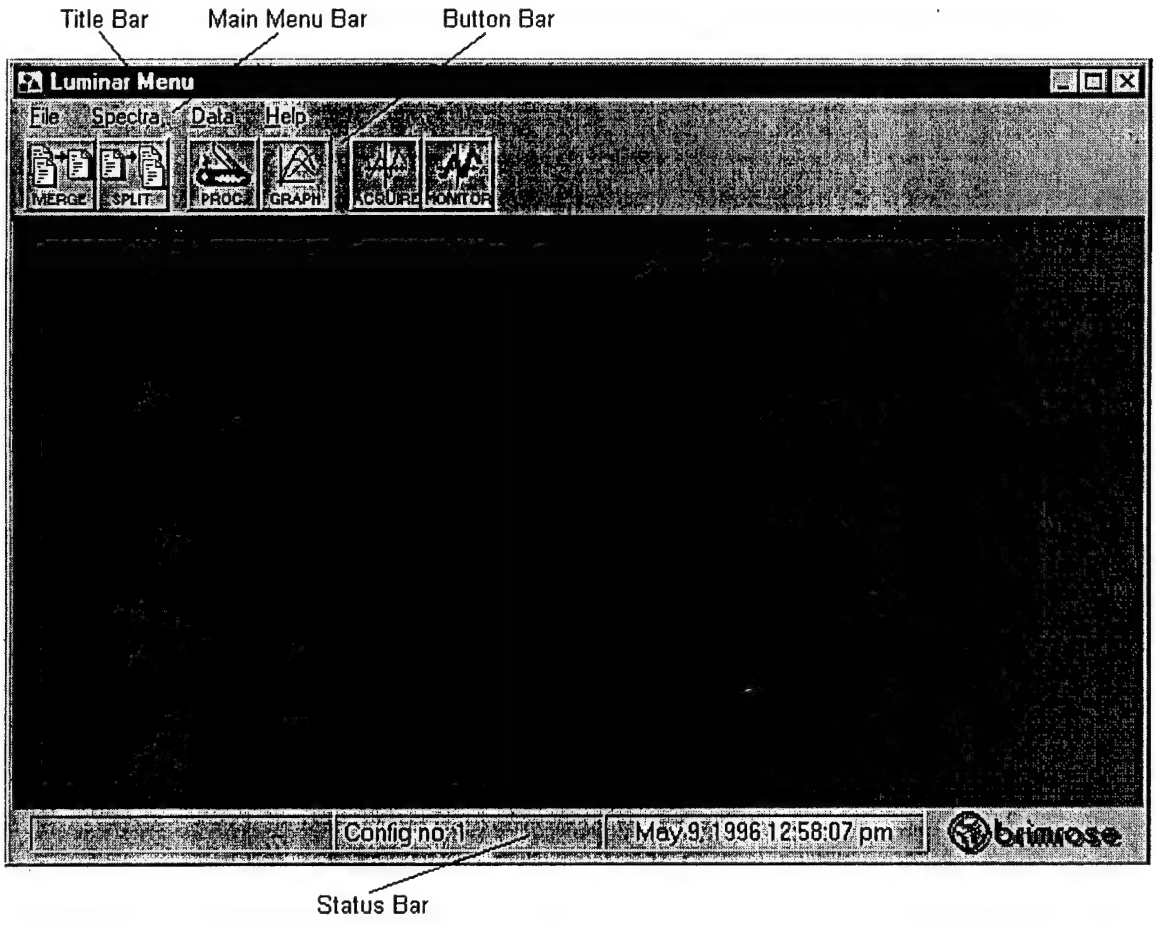
Windows program files

The Windows Luminar software package consists of several files that are necessary for the application to run properly. These files will be described below.

DDEMENU.EXE	Main program file.
LUMNET.CFG	Network configuration file. This file contains parameters necessary to configure the communication via TCP/IP stack. **It is analyzed internally by the communication software and should not be edited by the user**. One line, however, assigns the true Internet Protocol (IP) address to the spectrometer name. This line is factory set to LUM1=123.123.4.1. The last of the four numbers specifies the spectrometer number and can take values from 1 to 255. As a consequence, you can connect a maximum of 255 spectrometers to a network. **This line should not be changed unless you connect another spectrometer to the network**. However, if you do so, you should put the following line in the LUMNET.CFG file: LUM*x* = 123.123.4.*x* where *x* denotes the new spectrometer number.
DDEMENU.CFG	DDEMENU configuration file. This is a binary file containing the default parameters for all dialog windows.
DDEMENU.HLP	DDEMENU help file. This file contains descriptions for all functions and dialog boxes used in the menu program.
LUMSERV.EXE	The DDE server application. This program must run before DDEMENU is started. It acts as an intermediary between the communication software and the DDEMENU itself.
GRAPH.EXE	Data set graphing program. This program can be called from DDEMENU or used as an independent application. It allows to display and print spectral data sets in a 2-D or 3-D view.

Navigating in DDEMENU

DDEMENU uses a standard Windows graphical user interface and should be straightforward to anybody who used other Windows applications. The main application window is shown below.



The main application window contains three important elements. They are described below.

Main Menu Bar — The main menu contains four items: **File**, **Spectra**, **Data** and **Help**. Clicking on a main menu item will display a drop-down menu.
The File menu contains file processing commands (*Merge*, *Split* and *Edit*) as well as the *Exit* command. The **Spectra** drop-down menu contains commands for processing spectral data: *Chop*, *Average*, *Process*, *Translate* and *Graph*. The **Data** menu handles data acquisition and consists of two commands: *Acquire* and *Monitor*. Finally, the **Help** menu lets you access the program's help system and contains two commands: *Help* and *About*. The menu commands will be described in detail in the next sections of this manual.

Button Bar — The six buttons contained in the button bar let you quickly access some of the menu functions (*Merge*, *Split*, *Process*, *Graph*, *Acquire* and *Monitor*).

Status Bar — The left box contained in this line is used to display progress messages during acquire function. The box in the middle shows the currently used configuration number, and the right-side box shows the current date and time.

The best way to communicate with DDEMENU is to use the mouse to select menu options, click buttons and change layout of the windows. However, you can use keyboard in most operations, and some of them require you to do so (e.g., typing the file name).

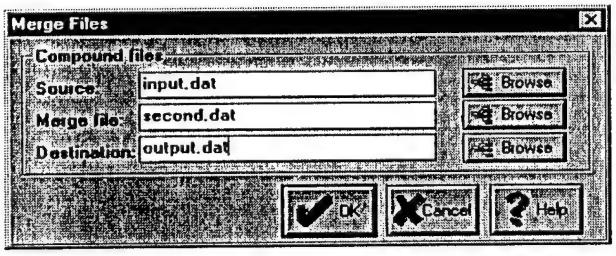
To select an item (e.g., a menu option), issue a command (e.g., click a button) or change the state of an item (e.g., unselect a check box), just click on the item with the left mouse button. You can also use *shortcut keys* to select items. To do so, press the **Alt** key together with the key corresponding to the highlighted letter.

Menus and options

Files

The **Files** menu contains four options: *Merge*, *Split*, *Edit* and *Exit*. These commands are used for file processing and viewing.

Merge

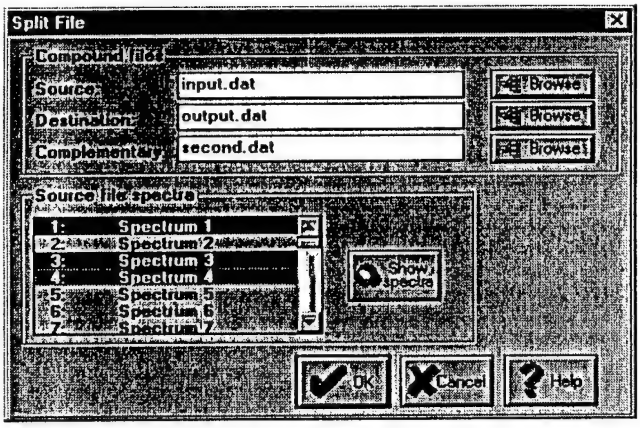


This function performs elementary file manipulation by taking two separate data files (*Source* and *Merge file*), each containing one or more spectra, and combining them into a single output file (*Destination*). The original data files are not changed. The output file will not be created if it already exists unless you confirm that the file may be overwritten. By pressing one of the **Browse** buttons, you can use a standard Windows Browse dialog box to find and specify a data file. For each file name there is a separate browse button. Use the **Tab** key or mouse to move the focus between the input boxes and buttons. The files will only be merged if the information contained in the headers of both input files is compatible, i.e., the starting and ending wavelengths, as well as the wavelength increment, must be the same.

To obtain a brief description of this function you may press the **Help** button. For each menu function the same **Help** button can be used to display the help window.

In the example shown above, the Merge *function will merge the files INPUT.DAT and SECOND.DAT and will create a new file, OUTPUT.DAT, when the* **OK** *button is pressed.*

Split

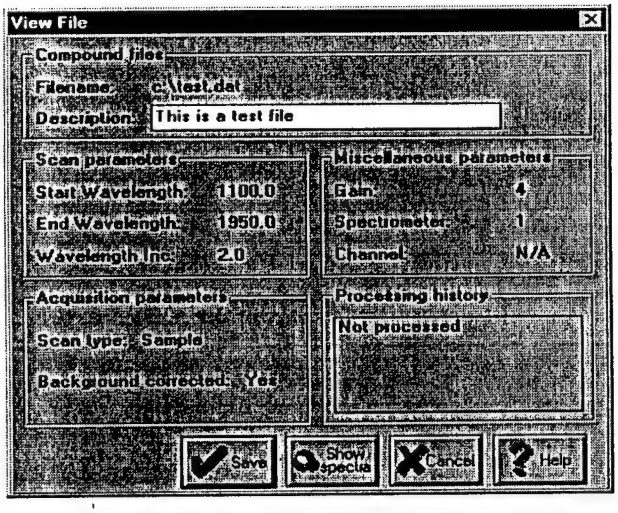


This dialog box allows you to manipulate data by taking a single input file and splitting it into two separate output files. The *Source* (original) data file is preserved. The *Source file spectra* list box is used to select the spectra that will be placed in the *Destination* file. The remaining entries in the *Source* file may be placed in a *Complementary* file if the name of the file is specified. Use the **Show spectra** button to refresh the spectra descriptions if the source file name has changed. As with other functions, if the *Destination* or *Complementary* files already exist, an appropriate message will be displayed to warn you.

The example above will take the first, third and fourth spectrum from the file INPUT.DAT, and place them in a new file called OUTPUT.DAT. The remaining spectra will be copied into a complementary file SECOND.DAT.

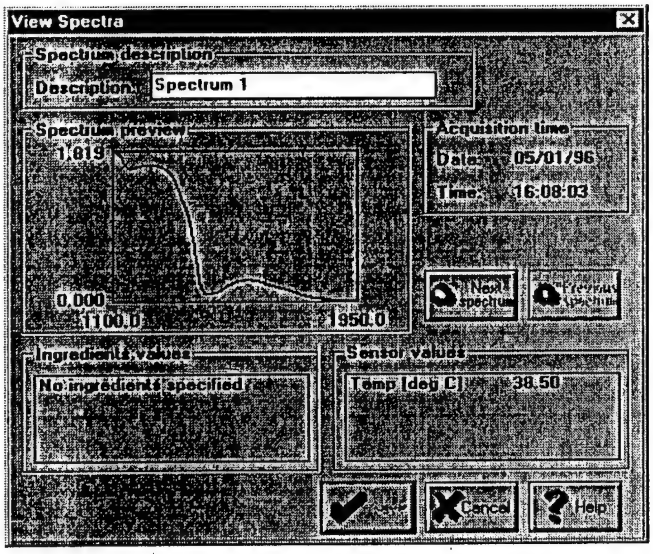
Edit

This command lets you view and edit data file characteristics. The selected file data are displayed in two dialog boxes:



The *View File* dialog box shows basic information describing the data file (name and description, scanning parameters, scan type and background

correction, post-processing operations performed). After you click the **Show Spectra** button, a second dialog box appears:



The *View Spectra* dialog box lets you view the parameters of an individual data file entry. The information contained in the box includes entry name and date/time of acquisition, spectrum preview, sample data and process sensor values. Use the **Next/Previous spectrum** buttons to select entries to view.

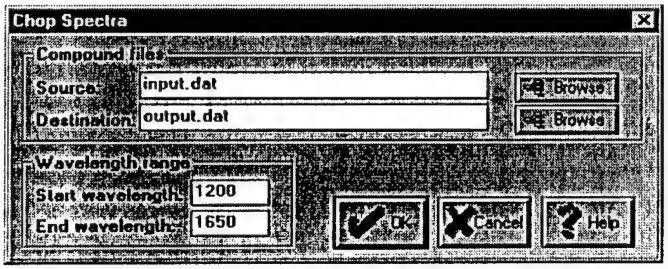
Exit

This command will exit the DDEMENU application. You can also exit DDEMENU by pressing **Alt-F4** or clicking the Close button located in the top right corner of the window.

Spectra

The **Spectra** menu commands let you process data contained in spectral files (*Chop*, *Average* and *Process*), export data to other file formats (*Translate*) and view spectra graphically (*Graph*).

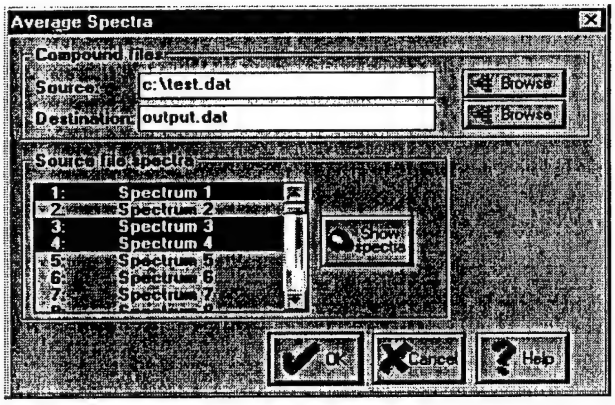
Chop



This function lets you truncate or "chop" the current wavelength range of a spectrum (or series of spectra) contained in the source file and copy the resultant data to the destination file. As before, the original data file is preserved and the output file is written only if it does not exist or you allow it to be overwritten. If the wavelength specified is outside of the wavelength range of the *Source* file a warning message will be displayed.

In the above example we assume that the data file named INPUT.DAT contains 35 spectra collected over the wavelength range of 900 to 1700 nm. The above command will create a compound file named OUTPUT.DAT, containing the same 35 spectra truncated to the wavelength range of 1200 to 1650 nm.

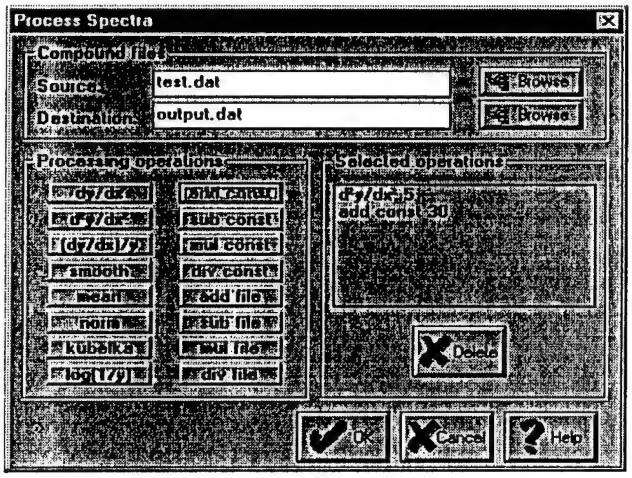
Average



This function takes a compound data file and averages the selected spectra into a single output file. You may use the **Show spectra** button to refresh the spectra descriptions in case the *Source* file name has changed. The input file remains unchanged and the output file will only be created if it does not exist or you confirm overwriting it.

In the example shown above, the function will take the first, third and fourth spectra out of the file TEST.DAT, average them and write the averaged spectrum into a new file named OUTPUT.DAT.

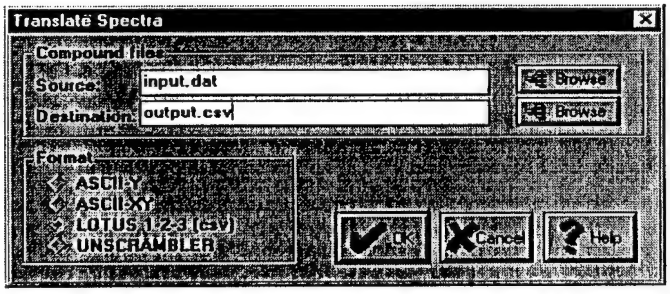
Process



This dialog box lets you process each spectrum contained in the *Source* file using one or several mathematical functions. By clicking the appropriate buttons in the *Processing operations* group, you can specify the operation to be performed. The selected operations appear in the list box on the right and are always executed in a top-down order. If an operation requires entering additional parameters, the appropriate dialog box will be displayed (for example, the **dx**, **d2x**, **dx/x** and **smooth** operations require the Savitsky-Golay smoothing value of 5, 7, 9, 11 or 13.) The **add**, **subtract**, **multiply by** and **divide by constant** or **file** functions require additional specification of a constant or file name. If you want to delete the previously selected operation, simply highlight it and click the **Delete** button.

For more information on post-processing operations, see Appendix (page 14).
In the above example, the input spectra contained in the file TEST.DAT will be processed with the second derivative (with a smoothing value of 5), and then the constant value of 30 will be added to each data point.. The resultant spectra will be placed in a file named OUTPUT.DAT.

Translate

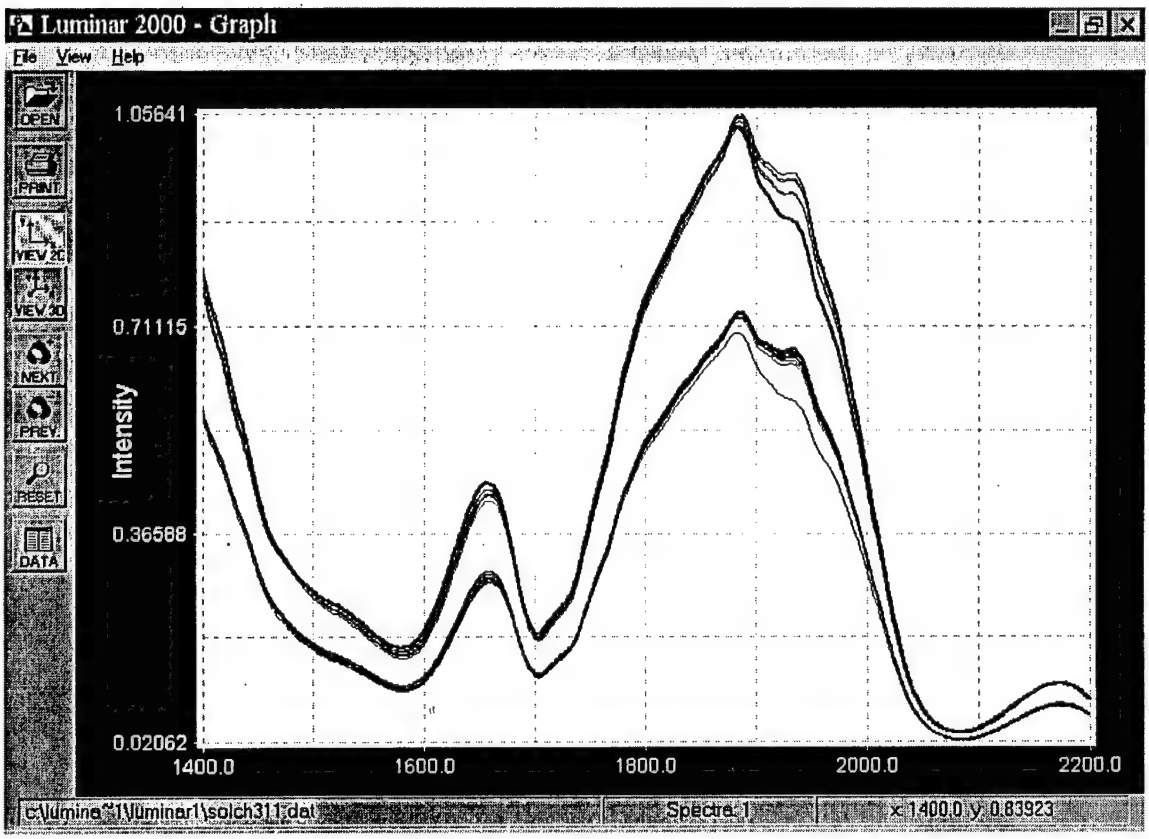


This command performs data translation from the Brimrose binary file format (described in the Appendix) to several other formats. The currently supported formats are ASCII Y or X-Y pairs, CSV (Comma Separated Variable) format (suitable for most spreadsheet programs) and The Unscrambler (DOS) format. The format is selected by clicking one of the radio buttons in the *Format* group box.

The example in the figure will read the binary file INPUT.DAT and export its contents to a file named OUTPUT.CSV in Comma Separated Variable format.

Graph

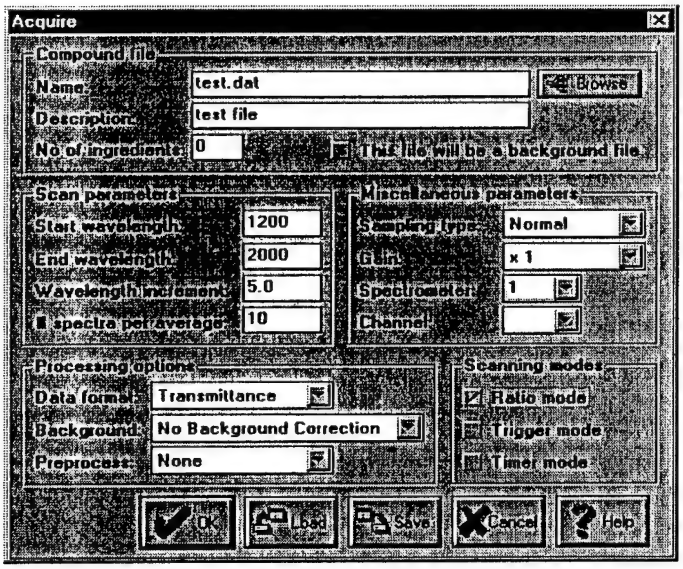
This command invokes the Graph program that allows you to view spectra graphically and print them.



Data

The Data menu contains data acquisition commands: *Acquire* and *Monitor*. The *Acquire* command is used to read spectral data from the spectrometer and put them in compound files. To monitor data without saving them, use the *Monitor* command.

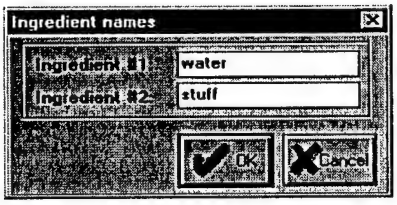
Acquire



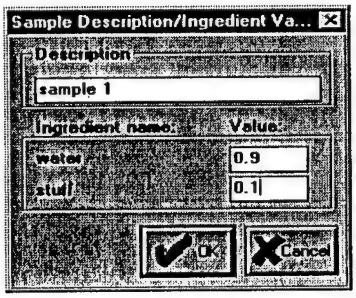
This function allows you to communicate with the Luminar spectrometer in order to acquire spectra and save them in the Brimrose Format data file. If you want to describe the sample for which spectrum was taken (for example, with concentrations for each ingredient), the *No of ingredients* parameter must be set to the number of the sample components. For each spectral scan, the concentration (or any other description) must be entered for each ingredient.

As a result, a compound file will be created. If the file already exists, you can either enter a new file name or overwrite the existing file. If the spectrometer is not switched on or communication problems occur, the *Spectrometer not responding* message box will be displayed. In case the spectrometer cannot perform a scan using the specified parameters, the *Parameters rejected* message will appear. The *Description* parameter specifies the data file main header description. By default it is set to "Compound file" but it can be changed to any string of no more than 24 characters.

After clicking the **OK** button you will be asked to describe the sample components (ingredients):



Before performing the scan, the program will also prompt you to enter the sample description, including ingredient concentrations:



However, if you specify no components (by entering "0" in the *No of ingredients* field), you will only be asked to enter the scan description (the default description is "Spectrum *n*" where *n* is the spectrum number starting from 1). The data received from the spectrometer are appended to the compound file and a small graph will be displayed in the top left corner of the screen.

The **Acquire** dialog parameters are described below.

Name — Specifies the name of the Brimrose Format data file in which the acquired spectra will be saved. Checking the *This file will be a background file* box will store the file in a background file format.

Description. — This field should be used to enter any description for the data file (up to 24 characters).

No of ingredients — Number of ingredients contained in the sample. Any sample can contain up to 10 ingredients. If this number is greater than zero, you must enter the ingredients names, and then, each time a spectra is taken, enter values for each ingredient.

Start wavelength, End wavelength — These values specify the starting and ending wavelength of the scanned interval in nanometers (inclusive). The minimum and maximum values depend on the spectrometer wavelength range.

Wavelength increment — Wavelength scanning increment. The minimum value is 0.3 nm.

 # spectra per average — Number of scans taken for averaging. The minimum value is 1.

Sampling type — Sampling mode. The following modes are available:

- *Sample* — the "simple" spectrum of a sample will be taken;
- *Internal Reference* — the scan will be taken from the internal spectrometer reference detector;
- *Internal Standard* — the scan will be taken from the internal reference detector with a polystyrene standard placed in its optical path.

Gain — Spectrometer internal amplifier gain. The permitted gain values are 1, 2, 4 and 8.

Spectrometer — Number of the spectrometer (if more than one spectrometer is available on the network). The number can vary from 1 to 255.

Channel — Optical multiplexer channel number. This field is disabled for single-channel systems (not equipped with the optical multiplexer).

Data format — Spectral data format. Available formats are transmittance and absorbance. This field is disabled if Ratio mode is not selected.

Background — This field lets you specify whether or not to divide the raw scanned data by the contents of a previously stored background file. The parameters of the spectrum (start, stop and increment) must be the same as those used to acquire the background file.
The list of available background files can be displayed by expanding the drop-down list box. If no files are available in the working directory or if Ratio mode is not selected, background correction will be disabled.

Preprocess — Optional spectrum preprocessing with the first or second derivative.

Ratio mode — Check this box if you want the incoming data in the relative form, i.e. divided by the values taken from the reference arm of the spectrometer. This feature will compensate for source drift of the spectrometer.

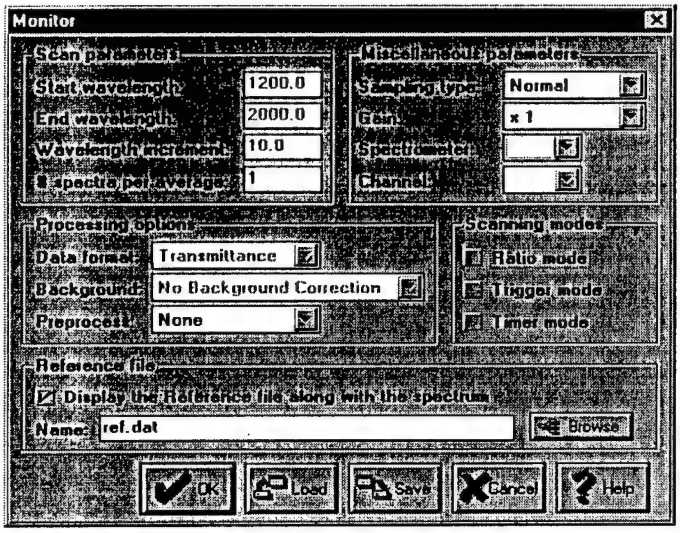
Trigger mode — Check this box if you want to trigger the scanning with an external hardware signal. This signal is provided by devices connected to the spectrometer **Trigger** input.

Timer mode — Checking this box allows you to run scans periodically every *n* seconds. The interval between scans is defined in a separate dialog box.

If you want to save the parameters for future use, click the **Save** button to write the current configuration into a file. Since there are five configurations available, you will be prompted to enter

the configuration number. Similarly, if you want to restore configuration parameters, click the **Load** button. Initial values of the parameters make up the current configuration (the number of the current configuration is displayed in the middle box on the main window status bar.

Monitor



This function is similar to the **Acquire** function in the way it communicates with the spectrometer. The difference is that the **Monitor** function does not allow to save the scanned data in a file. Therefore the **Monitor** dialog box does not contain the *Name*, *Description* and *Number of ingredients* fields which are not necessary for the execution. However, you can compare the actual data against reference data by displaying both spectra in the monitor window. To specify reference file, check the *Display the Reference file along with the spectrum* box and enter its name in the *Reference file Name* box. After pressing the **OK** button the program will enter a loop and will continuously acquire and display raw data using a graph window. To stop the loop, click the **Stop** button in the graph window or press the **Escape** key.

Help

Help

This function lets you access the program's help file to quickly find the information you need.

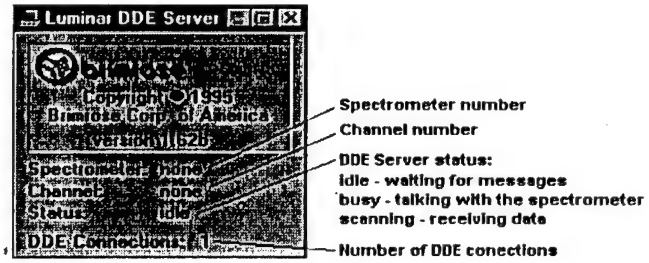
About

This command displays a brief program description box.

Luminar DDE Server

The LUMSERV.EXE application is the Windows Dynamic Data Exchange (DDE) server that can acquire data from the Luminar spectrometer via TCP/IP protocol, process it, and then send the data in binary or ASCII formats (such as National Instruments LabVIEW) to DDE clients. Any DDE client application can open a connection with the server and request data from the spectrometer. There can be up to 10 simultaneous DDE connections opened. The LUMSERV application connects to the Luminar spectrometer using the TCP/IP protocol during initialization and runs minimized. The server

can also run maximized; in this state it displays four parameters: current spectrometer and channel number, the last DDE command and the number of DDE connections.



Upon loading, the DDEMENU application will always try to load the DDE Server automatically. If this operation fails, an error message will be displayed.

Appendix

Post-processing operations

First Derivative

This function uses the Savitsky-Golay convolution function to obtain a smoothed first derivative of the data. The number of data points that will be used in the weighted smoothing can be 5, 7, 9, 11, or 13. For chemometric modeling, the first derivative is often applied to the data to emphasis changes in the data and to compensate for any baseline offsets from one spectrum to the next. The first derivative can degrade the signal to noise ratio.

Second Derivative

This function uses the Savitsky-Golay convolution function to obtain a smoothed second derivative of the data. The number of data points that will be used in the weighted smoothing can be 5, 7, 9, 11, or 13. The second derivative is often used to compensate for any changes in slope in the baseline in addition to baseline offsets (as the first derivative does). The use of the second derivative further decreases the signal to noise ratio.

Mean Center

Mean centering uses a two-step data processing. First an average spectrum is calculated from the individual spectra in the data file. This average spectrum is then subtracted from each individual spectrum. Mean centering emphasizes the subtle differences between the spectra. Since eigenvector-based chemometric methods (e.g. PCA, PLS) are based on changes in absorbance data, mean centering the data often leads to improvements in modeling them.

Kubelka-Munk

The Kubelka-Munk (KM) algorithm is used in diffuse reflectance to convert reflectance data into KM units that are linear with repsect to concentration. The KM algorithm requires two single beam spectra and is represented by the following equation:

$$KM = \frac{\left(1 - \frac{Sample\ File}{Background\ File}\right)^2}{2 \times \left(\frac{Sample\ File}{Background\ File}\right)}$$

Smooth

The smoothing function uses the Savitsky-Golay convolution function to smooth, or remove, random noise in the spectrum. The Savitsky-Golay algorithm uses a weighted average of points to calculate a smoothed data point. The number of data points that will be averaged together can be 5, 7, 9, 11, or 13. Choosing the value of 5 will provide less smoothing while choosing the value of 13 will provide the most smoothing (and the resultant loss of some fine details in the spectrum).

Normalize

This processing function scales all the spectrum such that the maximum value is one and the minimum value in the spectrum is zero. Normalization is often used to simplify visual interpretation of spectra.

Add constant
Subtract constant
Multiply by constant
Divide by constant

These operations let you modify spectra by applying a constant value to each point. They can be used for background correction, amplifying the spectrum values, and so on.

Add file
Subtract file
Multiply by file
Divide by file

These operations let you combine two sets of spectral data (contained in two separate files) by adding, subtracting, multiplying or dividing the values of corresponding points. They can be used for bakcground correction, enhancing spectrum shape through nonlinear rescaling, and so on.

Brimrose binary file format

The DDEMENU.EXE program stores the spectral data in a proprietary binary format. This approach minimizes the volume of data files while keeping the precision unaffected; however, transfering data to other software packages requires special translation utilities that can change the file format to match the target application. The translation operation is handled by the Translate command in the Spectra menu; currently supported formats include ASCII-Y or XY pairs, Comma Separated Variable (CSV) and The Unscrambler. Moreover, translation software is provided by InfoMetrix and Galactic Industries that converts Brimrose files into Pirouette and Grams-386 data files. This provides you with the ability to move the acquired data into a number of software platforms and should guarantee enough flexibility for most cases. In addition, however, Brimrose provides both a description of its file format and the C code header files to enable you to write your own software to read the spectral data directly into your own programs. Figure A-1 below shows the general layout of the data file, while the following C language structures outline the actual contents of both the main- and sub-header.

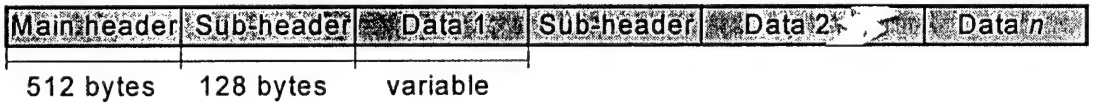


Figure A-1 *Brimrose Format data file layout*

A basic single-spectrum data file consists of three distinct portions: the main header, the subheader and the data section. The main header contains general information including description field, data collection conditions, date and time, and optional description of the sample components. The sub-header contains quantitative information describing the chemical constituents comprising the sample. This information is typically used during a chemometric calibration phase. The sub-header also contains a description field. The data are stored as an array of 4-byte floating point numbers. Data sets can also be compound, i.e. contain more than one spectrum. In this case the file consists of a main header with subsequent sub-headers/data sections. The number of discrete spectral data sets in a compound file is contained in the main header. The information contained in the main header pertains to all subsequent spectra in the data set, while each sub-header refers only to the data immediately following it in the file.

The C language structures describing the layout of the main header and sub-header are shown below.

Main header layout:

```
typedef struct
{
  long   id;                    // file ID = 195498812L
  char   descr[DESCR_LENGTH];   // file contents description (<25
characters)
  float  start,                 // start wavelength
         stop,                  // end wavelength
         increment;             // wavelength increment
  unsigned short numpoints,     // number of points in spectrum
         numscans,              // number of scans per average
         entries,               // total number of entries in file
         gain;                  // ADC gain (now 1-2-4-8)
  char   scantype,          // SAMPLESCAN, REFSCAN, POLYSCAN or BACKSCAN
         axis,              // wavelength axis units (AXIS_NM or AXIS_RF)
         node,                  // spectrometer number (1-255)
         channel,               // channel # (0 - no multiplexer)
```

```
        preproc;                // preprocessing type
  unsigned short prelist[MAX_PROCS]; // preprocessing ID table
  char   ings;                  // number of components in file
  char   ingname[MAX_INGS][ING_NAME_LENGTH];   // component names
  char   sensors;               // number of process parameters
  char   sensorname[MAX_SENSORS][SENSOR_NAME_LENGTH];  // parameter names
  char   filler[56];            // future expansion
} MAINHEAD;                     // total header size = 512 bytes
```

Sub-header layout:

```
typedef struct
{
  time_t scanid;                // scan date and time
  char   descr[DESCR_LENGTH];   // spectrum description
  float  ing_vals[MAX_INGS];    // component quantities
  float sensor_vals[MAX_SENSORS]; // parameter quantites
  char   filler[3];             // future expansion
} SUBHEAD;                      // total header size = 128 bytes
```

DDE communications using the DDE Server

The Luminar DDE server registers the Service Name as "Luminar". The Topic Name for all Items is "Server".

The Item Names are described below.

SetParams Sets the scan parameters. The parameters are listed below.

Start=xxx.x	start wavelength.
Stop=xxx.x	stop wavelength.
Inc=xxx.x	wavelength increment (default 1.0).
Avgs =xxx	number of averages (default 10).

Type=x	scan type:
	x = 0 sample scan (default);
	x = 1 internal standard;
	x = 2 internal reference.

Gain=x	spectrometer internal gain; x = 1, 2, 4, 8 (default = 1)

Units=x	units of measure used when data returned:
	x = 0 nm (default);
	x = 1 MHz;

Ratio=x	ratio mode: x = 0 ratio off (default); x = 1 ratio on.
Spectr=x	spectrometer number (1–255, default = 1).
Channel=x	channel number (1–255 or 0 if no optical multiplexer is present).
Preproc=x	signal preprocessing: x = 0 don't process (default);

x = 1 process with first derivative;
x = 2 process with second derivative.

Filter=x Savitsky-Golay smooth value (range 1–5, default = 1).

Form=x data format:

x = 0 use raw data format (default);

x = 1 transforms using the equation

$$\text{Data} = \log_{10}(\frac{1}{Y})$$

x = 2 transform to transmittance units:

$$T = \frac{Y}{BackgroundData}$$

x = 3 transform to absorbance units:

$$A = \log_{10} \frac{BackgroundData}{Y}$$

Trig=x triggering mode:

x = 0 don't use trigger (default);

x = 1 use trigger.

In order to set several parameters in one DDE Poke command the parameters should be separated with semicolons (;).

For example, the string Start=1200;Stop=1600;Inc=1.0;Avgs=10 *sent with the* ***SetParams*** *command will set four parameters: start wavelength, stop wavelength, wavelength increment and number of averages. The other parameters will be set to their default values.*

SetPass Sets the password to be used when communicating with the spectrometer.

SetFile Sets the file name of the file that will receive the data while executing the **SaveSpectra** command.

ScanSpectra Starts the scan and returns the data in TEXT or BINARY format.

SaveSpectra Starts the scan, returns the data, and saves them in a Brimrose Format data file. The file name must be previously set using the **SetFile** command. If the file of this name already exists and the start wavelength, stop wavelength and wavelength increment values are identical with the scanned spectra, the **SaveSpectra** command will append the data to the end of the file and the file's main header will be updated. If the existing file is not a data file, it will be overwritten.

SaveRef Starts the scan, returns the data, and saves them in the background data file named BAK.DAT.

The data are saved in a null-terminated-string format (i.e. a string of characters ending with a \0, or NULL character), compatible with LabVIEW and many other applications. The data string is composed of the following elements:

 # points a string representing a 16-bit integer number in range of 0..32767;

Point(s) a string of tab-separated numbers representing 32-bit floating point values from the range of $8.43*10^{-37}..3.37*10^{38}$ (positive or negative).

Example:

Consider the following data string:

"3\t12345.6789\t0.0\t98765.4321\0"

There are three (as determined by the first character) samples in the data string:

first sample	12345.6789
second sample	0.0
third sample	98765.4321

The \t symbol represents the Tab character (ASCII 9), and the \0 symbol represents the NULL character (ASCII 0).

If an error occurs during scan, the number of elements in the sample is 0, –1 or –2 depending on the error. Such a sample does not contain any data points. The error codes are:

0: spectrometer not responding.

–1: parameters out of range.
–2: error writing to a file

7.0 REFERENCES

1. Xiaolu Wang, Acousto-optic tunable filters spectrally modulate light, Laser Focus World, Vol. 28, No. 5, PP. 173-180, (1992)

2. AOTF spectroscopy: A powerful new tool in near infrared spectroscopy, Application Note 410, Brimrose Corp., (1992).

3. I.C. Chang, Tunable Acousto-Optic Filter Utilizing Acoustic Beam Walkoff in Crystal Quartz," Appl. Phys. Lett., **25**, 323-4, (Sept. 1974).

4. P. Katzka, "AOTF Overview: Past, Present and Future," SPIE **753**, 22, (1987).

5. J. Xu and R. Stround, "Acousto-optic devices," John Wiley & Sons, Inc., (1992).

6. L. T. Nguyen and C. S. Tsai, "Efficient wideband guided-wave acousto-optic Bragg diffraction using phased surface acoustic wave in $LiNbO_3$ waveguides," Appl. Opt., Vol. 16, PP. 1297-1304, (1977).

7. K. Higuchi, K. Yamanouchi and K. Shibayama, " TE-TM mode conversion by interaction between elastic surface wave and laser beam on a metal-diffused optical waveguide," Appl .Phys. Lett., Vol. 28, PP. 75-77, (1976)

8. R. Opitz, V.P. Hinkov and W. Sohier, " Collinear acousto-optical TM-TE mode conversion in proton exchanged Ti-LiNbO3 waveguide," J. of Lightwave Tech., 903-908, (1988).

9. I. C. Chang, "Collinear beam acousto-optic tunable filters," Electronics Lett. Vol 28 No 13 PP 1255-1256 (1992)